GENETICS

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BEARING ON HEREDITY AND VARIATION

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TABLE OF CONTENTS

NEWCOMBE, HOWARD B., AND GRACE WORKMAN SCOTT, Factors responsible for the delayed appearance of radiation-induced mutants in *Escherichia coli* . . 475

HOULAHAN, MARY B., G. W. BEADLE AND HERMIONE GRANT CALHOUN, Linkage studies with biochemical mutants of *Neurospora crassa* 493

CRARY, D. D., AND P. B. SAWIN, Morphogenetic studies in the rabbit. VI. Genetic factors influencing the ossification pattern of the limbs 508

BROWN, WILLIAM L., Numbers and distribution of chromosome knobs in United States maize 524

WHITE, M. J. D., A cytological survey of wild populations of Trimerotropis and Circotettix. (Orthoptera, Acrididae). I. The chromosomes of twelve species 537

GREEN, M. M., A study of tryptophane in eye color mutants of Drosophila . 564

GARDNER, ELDON J., AND CHARLES M. WOOLF, Maternal effect involved in the inheritance of abnormal growths in the head region of *Drosophila melanogaster* 573

IRWIN, M. R., On immunogenetic relationships between the antigenic characters specific to two species of Columbidae 586

PERKINS, DAVID D., Biochemical mutants in the smut fungus *Ustilago maydis* . 607

STEPHENS, S. G., The cytogenetics of speciation in Gossypium. I. Selective elimination of the donor parent genotype in interspecific backcrosses . . . 627

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FACTORS RESPONSIBLE FOR THE DELAYED APPEARANCE OF RADIATION-INDUCED MUTANTS IN ESCHERICHIA COLI

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THAT mutations are produced in bacteria following treatment with X-rays and ultra-violet light has been shown by a number of workers (GRAY and TATUM 1944; GOWEN 1941; ROEPKE, LIBBY, and SMALL 1944; and TATUM 1945, 1946). However, where irradiated bacteria have been tested for the presence of induced mutants immediately after treatment few have been found, and detailed studies show that appreciable growth must take place in the treated population before the bulk of the induced changes appear (DEMEREC 1946; DEMEREC and LATARJET 1946).

The proportion of the total mutations which are delayed in this manner amounts to 99 percent or more in the one case studied (mutation of *E. coli* strain *B/r* to resistance to bacteriophage *T1*); and the population increase required in order for all of them to appear is approximately 2^{12} to 2^{13} times.

This "delayed effect" of irradiation is similar in both X-ray and ultra-violet treated material, and parallel phenomena have recently been demonstrated following exposure to nitrogen mustard (BRYSON 1947), sodium desoxycholate and acriflavine (WITKIN, personal communication).

The underlying cause of the delayed appearance of mutants, could be either (a) induced gene instability causing an increase in the spontaneous mutation rate over a number of cell generations, or (b) induced gene mutation requiring one or more cell divisions in order to affect the phenotype of the organism.

The first of these two interpretations (gene instability) assumes an effect of radiation which has not been observed in higher organisms, and thus implies a fundamental peculiarity of bacterial genes. However, gene instability lasting over many cell generations has been produced in Drosophila by the action of mustard-gas (AUERBACH 1947) and it is conceivable that a similar effect is produced in bacteria by irradiation (see PONTECORVO 1946).

The alternative interpretation assumes delayed phenotypic expression, an effect already demonstrated in a class of spontaneous mutations of *E. coli* (resistance to phage *T1*, NEWCOMBE 1948). However, spontaneous mutations require only two or three generations in order to become detectable, and this would seem inadequate to explain more than a part of the prolonged delay associated with induced mutation.

The two interpretations raise a number of points of fundamental importance:

(a) If there is a delay in the phenotypic expression of gene mutation, the evidence is relevant to the general problems of gene action and cell physiology.

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(b) If radiation induces gene instability in the bacteria, there must be a fundamental difference between the genes of this organism and those of higher forms. This would be of evolutionary significance.

(c) On the other hand, if bacterial genes are identical to those of higher forms the general knowledge of the mutation process can be advanced, since this organism promises to yield quantitative information which would be unobtainable with larger and more slowly breeding materials (See LURIA 1946 for a discussion of the point).

For these reasons an attempt was made in the present study to discriminate between the two interpretations of the "delayed effect"

DELAYED APPEARANCE OF INDUCED MUTANTS

The available information concerning the delayed effect of irradiation and the further data required in order to discriminate between the alternative interpretations will now be considered.

The effect was first demonstrated by DEMEREC (1946) who adopted the following procedure: suitable numbers of irradiated cells were spread on the surface of nutrient agar and incubated for varying periods of time; at the end of this they were sprayed with phage *T1* to eliminate all susceptible bacteria, and incubated further until colonies of visible size appeared. Estimates of population increase during the various periods of growth prior to spraying were made by dilution and plate count, using samples from a broth culture of the treated bacteria which had been incubated at the same time

Since the descendents of a treated bacterium are confined to one locality on the agar, a single colony will result from the presence of a resistant cell in a clone. Each resistant colony indicates that a mutation has taken place.

However, the method does not indicate the proportions of resistant and susceptible cells descended from individual treated bacteria, and this information is necessary if we are to decide whether the induced gene mutations took place prior to growth or after one or more cell divisions.

Furthermore, it is conceivable that the treated cells giving rise to resistant mutants do not start to divide until some time after the population as a whole has entered the exponential growth phase. If this is the case one can obtain only a maximum estimate of the number of cell generations required for the appearance of the induced mutants.

Further information is therefore necessary, and its nature will be evident from a consideration of the genetic consequences of the two possible causes, either acting alone or in conjunction with a delay in the onset of division. These are illustrated in figure 1 as follows:

(A) delayed mutation,

(B) delayed mutation plus delayed onset of division,

(C) delayed phenotypic expression, and

(D) delayed phenotypic expression plus delayed onset of division.

In this figure the mutation is represented as appearing after a population increase of 2^4 times. Also, it has been assumed that there is only one gene complement in the cell and that each gene behaves as a single unit toward the

irradiation. Other assumptions could have been chosen, but a consideration of them will be left until the discussion.

Experiments to discriminate between these interpretations will be considered in the subsequent sections.

MATERIALS AND METHODS

Escherichia coli strain *B/r* (WITKIN 1946, 1947) and phage *T1* (DEMEREC and FANO 1945) were used throughout these experiments. The mutations studied were those resulting in the development of resistance to phage *T1*, that is from *B/r* to *B/r/1* and *B/r/1,5*. No attempt was made to distinguish between these two categories of mutation, and throughout the paper all *T1* resistant mutants will be referred to collectively as *B/r/1*.

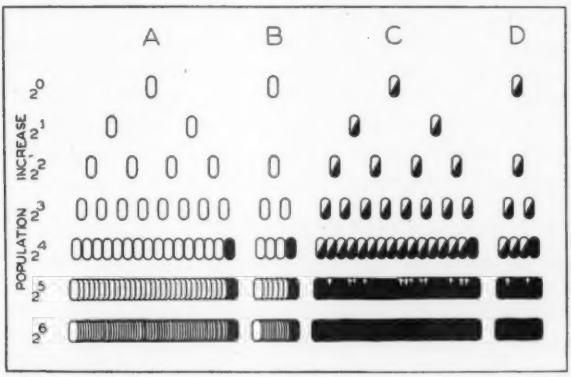

FIGURE 1.—Illustrating the genetic consequence of:
(A) delayed mutation (due to induced gene instability)
(B) delayed mutation plus delayed onset of division
(C) delayed phenotypic expression
(D) delayed phenotypic expression plus delayed onset of division.

Note: White figures represent non-mutants, white-black figures represent mutants which have not yet developed phenotypic phage resistance, and black figures represent phage resistant mutants.

Cultures of *B/r* were grown to saturation in a medium transparent to ultra-violet light (the M9 synthetic medium of ANDERSON 1946). Undiluted suspensions containing approximately 15×10^8 bacteria per cubic centimeter were irradiated with a General Electric Germicidal Lamp, this being estimated to deliver 95 percent of the ultra-violet energy in the 2,537 Ångstrom line. For irradiation, 10 cc quantities of the suspension were placed in Petri dishes of 100 mm diameter and shaken mechanically in order to ensure uniform exposure of all cells. The frequency and amplitude were such as to produce standing waves which did not quite reach the point of splashing.

A series of exposures was made ranging from one minute upward at a distance of 41 centimeters, the intensity being 1,600 ergs $\times$ millimeter$^{-2} \times$ min-

utes^{-1} as measured by a Westinghouse ultra-violet meter.

Numbers of viable bacteria were determined throughout the experiments by dilution and plate count. Numbers of phage resistant mutants were determined in a similar manner using agar plates which had previously been spread with phage *T1*.

EVIDENCE FROM THE ESTIMATED TIMES OF APPEARANCE OF ACTIVELY DIVIDING MUTANT CLONES

Of the interpretations discussed earlier, C (figure 1) is the one most readily tested. This assumes that mutant clones are present as actively dividing entities from the time when the treated population first begins to multiply, and that the delay is a matter of the number of cell generations required for phenotypic expression, this ranging from zero up to 12 generations.

Information on this point can be obtained from the sizes of individual mutant clones in fully grown cultures provided that mutants and non-mutants divide at equal rates, an assumption which will be tested later. Thus, where n_1 is the number of viable bacteria in the inoculum, n_2 the number in the fully grown culture, and m_2 the number of cells from a single mutant clone at the end of growth, the time at which the mutant clone appears as an actively dividing entity can be determined from the formula

$$g = \log(n_2/n_1 \cdot m_2)\log 2$$

Where all the cells of the inoculum have started to divide at approximately the same time, g will represent the number of cell generations occurring prior to the appearance of the dividing mutant clone. Where, on the other hand, the cells of the inoculum have started to divide at widely different times one cannot, of course, speak in terms of cell generations in the culture as a whole, but the population of the culture at the time when the mutant clone appeared will have been approximately 2^g. This latter estimate of the population will not be strictly accurate since it takes no account of the numbers of viable cells which start to divide after the appearance of the mutant clone. It will, however provide a close approximation except in the very early stages of the growth of the culture before the numbers of cells in the actively dividing part of the population appreciably exceed the number which are still in the lag phase.

In interpretation C, which we are considering in this section, the value of g should be zero in the case of all mutant clones, whereas in interpretations A, B, and D it would be expected to vary for different mutations from zero up to approximately 12.

To test this, series of cultures were grown from treated bacteria, the numbers of cells in the inocula being adjusted so that induced mutant clones would occur in only one out of every eight to ten cultures. Thus, the cultures in which an induced mutant clone developed would rarely contain more than one.

Material from the one minute and two minute exposures were used in these experiments, and 480 cultures were grown from each. Forty-six cultures from the one minute treatment and 57 from the two minute treatment contained

induced mutant clones in which the value of g was 10.5 or less. Those with values of g higher than 10.5 were ignored since an appreciable number would be expected due to spontaneous mutation alone.

The data from these experiments are presented in tables 1 and 2, and in histogram form in figure 2. The results of a similar control experiment, using a sample of the same bacterial culture which had not been irradiated, are given in table 3, and are also included in the histograms of figure 2.

TABLE 1

Delayed appearance of actively dividing mutant (B/r/1) clones following one minute irradiation with ultra-violet light. ($m_2/50$=number of resistant bacteria in a 0.1 cc sample of a fully grown test culture. g=estimated number of cell generations between irradiation and the appearance of an actively dividing mutant clone.)

EXPERIMENT	A		B		C		D	
No. of test cult.	120		120		120		120	
Vol. of test cult., cc	5		5		5		5	
Av. bact. in inoc.	98		71		25		45	
Av. end no. of bact.	11.3×10^8		11.7×10^8		11.6×10^8		13.5×10^8	
Cult. with g<10.5	11		14		12		9	
CULTURES	$m_2/50$	g	$m_2/50$	g	$m_2/50$	g	$m_2/50$	g
1	1,270,000*	—	790,000*	—	410,000	1.2	200,000	1.3
2	140,000	0.7	730,000*	—	228,000	2.2	140,000	1.9
3	85,600	1.4	410,000	0.0	160,000	2.5	87,100	2.6
4	18,120	3.7	114,700	1.5	116,500	3.0	28,300	4.2
5	8,400	4.8	29,800	3.5	102,800	3.2	24,100	4.4
6	7,200	5.0	24,800	3.7	56,500	4.0	15,200	5.1
7	4,510	5.7	15,300	4.4	52,100	4.2	1,161	8.7
8	3,730	6.0	12,200	4.8	37,500	4.8	1,100	8.9
9	3,230	6.2	9,100	5.2	12,000	6.3	757	9.1
10	3,120	6.3	8,900	5.2	9,400	6.6	—	—
11	498	9.3	2,170	7.3	947	9.9	—	—
12	—	—	1,090	8.2	877	10.1	—	—
13	—	—	724	8.8	—	—	—	—
14	—	—	713	8.8	—	—	—	—

* Numbers of resistant bacteria larger than required to give a value of g equal to zero.

Values of g were obtained which ranged from zero upward, and which might well exceed the value of ten if induced mutations could be detected against the rising background of spontaneous mutations.

The very small number of genotypically mutant clones which appear at the onset of growth suggest that delayed phenotypic expression alone cannot account for the "delayed effect." However, in order to estimate the times at which mutant clones appear it has been assumed that mutants and non-mutants increase at the same rate. Experiments to test this assumption are reported in the next section.

TABLE 2

Delayed appearance of actively dividing mutant (B/r/1) clones following two minutes irradiation with ultra-violet light. ($m_2/50$=number of resistant bacteria in a 0.1 cc sample of a fully grown test culture. g=estimated number of cell generations between irradiation and the appearance of an actively dividing mutant clone.)

EXPERIMENT	A		B		C		D	
No. of test cult.	120		120		120		120	
Vol. of test cult., cc	5		5		5		5	
Av. bact. in inoc.	311		247		205		193	
Av. end no. of bact.	12.0×10^8		9.5×10^8		10.5×10^8		9.3×10^8	
Cult. with g<10.5	22		8		12		15	
CULTURES	$m_2/50$	g	$m_2/50$	g	$m_2/50$	g	$m_2/50$	g
1	45,300	0.8	36,700	1.1	24,200	2.1	100,000	0.0
2	28,500	1.4	4,400	4.1	11,200	3.2	23,600	2.8
3	26,800	1.5	1,613	5.6	5,000	4.4	3,200	4.9
4	17,400	2.2	1,502	5.7	4,300	4.6	3,000	5.0
5	13,700	2.5	1,311	5.8	2,037	5.6	2,800	5.1
6	10,200	2.9	776	6.6	1,750	6.8	1,680	5.8
7	8,300	3.2	78	9.9	656	7.3	1,200	6.3
8	5,400	3.8	70	10.1	449	7.8	1,184	6.3
9	4,300	4.2	—	—	353	8.2	826	6.9
10	3,350	4.6	—	—	254	8.6	499	7.6
11	2,100	5.2	—	—	232	8.8	385	8.0
12	1,039	6.2	—	—	122	9.2	252	8.6
13	609	7.0	—	—	—	—	217	8.8
14	594	7.1	—	—	—	—	120	9.6
15	574	7.1	—	—	—	—	88	10.2
16	338	7.8	—	—	—	—	—	—
17	297	8.0	—	—	—	—	—	—
18	91	9.7	—	—	—	—	—	—
19	76	10.0	—	—	—	—	—	—
20	62	10.0	—	—	—	—	—	—
21	54	10.5	—	—	—	—	—	—
22	54	10.5	—	—	—	—	—	—

Competitive growth of mutant and non-mutant strains

The results presented in the previous section could arise from wide differences in the rates of increase of mutant and non-mutant strains when in competition. It is therefore necessary to determine whether differences of the required magnitude do in fact exist.

For such a test to be critical it is necessary (a) that the conditions of growth be identical to those in the original experiment, (b) that the mutant strains will not have undergone further change which would alter their ability to compete with non-mutants, and (c) that a number of independent mutant strains be tested.

(a) In the present tests particular attention was paid to the following conditions: (1) culture size, (2) size of the inoculum (and thus the period of

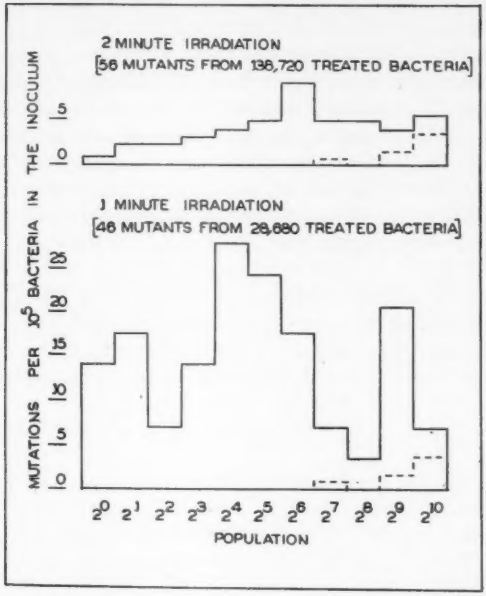

FIGURE 2.—Estimated times of appearance of ultra-violet induced mutations as actively dividing mutant clones. Time is expressed in terms of general population increase. Mutant clones from control experiment are indicated by broken lines. (From tables 1, 2, and 3.)

TABLE 3

Control experiment. Time of appearance of actively dividing mutant (B/r/1) clones in cultures from unirradiated cells. ($m_2/50$=number of resistant bacteria in a 0.1 cc sample of a fully grown test culture. g=estimated number of cell generations between resting stage, and the appearance of an actively dividing mutant clone.)

EXPERIMENT	A	
No. of test cult.	120	
Vol. of test cult., cc	5	
Av. bact. in inoc.	1154	
Av. end no. of bact.	11.5×10^8	
Cult. with $g<10.5$	8	
CULTURES	$m_2/50$	g
1	131	6.6
2	40	8.1
3	33	8.4
4	18	9.0
5	18	9.0
6	16	9.3
7	15	9.4
8	15	9.4

growth required to reach saturation), and (3) ratio of mutants to non-mutants in the inoculum.

(b) Inocula for the tests were taken directly from experimental cultures which contained induced mutant clones. This avoided the possibilities of change and selection inherent in isolating the mutant, freeing it from phage, and re-mixing with a non-mutant strain, a procedure which has been necessary when making similar tests with spontaneously occurring mutants.

(c) Resistant clones from six independent mutations were tested. Two of these were estimated to have appeared early in the growth of the cultures ($g=0$), two at an intermediate time ($g=6$), and two late in the growth of the cultures ($g=9$).

Two separate procedures were used. In the case of the early appearing mutant clones, ten or more cultures were grown from inocula containing approximately 700 bacteria, of which about 40 were resistant mutants. Any change in the proportion of mutants resulting from differences in the rate of increase was expressed as

$$m_2/n_2 \times n_1/m_1$$

where, n_1 and n_2 are the total numbers of bacteria, and m_1 and m_2 the numbers of resistant cells in the inoculum and in the fully grown test cultures. Ten or more independent single-plate estimates were made of each of these four values, those of n_2 and m_2 being each from a different test culture. Independent

TABLE 4

Change in the proportion of mutants (B/r/1) to non-mutants (B/r) as a result of competitive growth using induced mutant clones in which the estimated value of g is zero.

EXPERIMENT	A	B
No. of test cult.	17	10
Vol. of cult., cc	5	5
Av. bact. in inoc. (n_1)	707	719
Av. end no. bact. (n_2)	12.7×10^8	14.2×10^8
Av. mutants in inoc. (m_1)	47	40
Av. end no. mutants (m_2)	7.5×10^7	9.3×10^7
Generations growth	20.4	20.6
Av. $m_2/n_2\times n_1/m_1$	$0.94\pm.26$	$1.20\pm.40$

estimates of the value $m_2/n_2\times n_1/m_1$ were thus obtained for each test culture. Averages of these and standard deviations are given in table 4 together with other experimental data.

This procedure could not be used in the case of experimental cultures containing smaller proportions of resistant bacteria (those in which the mutant clones were estimated to have appeared later in the growth of the cultures). Inocula from these, in order to have a sufficient number of resistant cells to minimize statistical fluctuations in the value of m_1, would have in addition

very large total numbers of bacteria. This in turn would decrease the period of growth required to reach saturation, and thus might tend to obscure differences in the rates of growth of mutant and non-mutant strains. To overcome the difficulty small inocula were used, containing on the average one resistant mutant in every eight to ten instances. Large numbers of cultures (60 to 120) were grown, and the value $n_1/m_1 \times m_2/n_2$ determined for those which had received a resistant mutant in the inoculum, m_1 being assumed to be unity in all cases. No correction was made to compensate for the occasional occurrence of

TABLE 5

Change in the proportion of mutants (B/r/1) to non-mutants (B/r) as a result of competitive growth, using induced mutant clones in which the estimated value of g is 6 in experiments A and B, and 9 in experiments C and D.

EXPERIMENT	A	B	C	D
No. of test cult.	120	120	60	60
No. with mutant in inoc.	15	27	7	9
Vol. of cult., cc	5	5	5	5
Av. bact. in inoc. (n_1)	463	1280	2030	1770
Av. end no. bact. (n_2)	8.5×10^8	13.2×10^8	9.2×10^8	10.6×10^8
Assumed no. of mutants in inoc. (m_1)	1	1	1	1
Av. end no. of mutants (m_2)	3.0×10^6	2.8×10^6	3.2×10^6	1.7×10^6
Generations of growth	20.5	19.7	18.6	19.0
Av. $m_2/n_2 \times n_1/m_1$	$.16\pm.05$	$.32\pm.19$	$.78\pm.44$	$.28\pm.09$

two resistant mutants in an inoculum, since the magnitude of any bias from this source is negligible for present purposes.

The four mutant clones estimated to have appeared later in the growth of the experimental cultures ($g=6$ and $g=9$), have all been treated in this manner. Average values of $n_1/m_1 \times m_2/n_2$ are given in table 5, together with standard deviations.

If one is to interpret the varying numbers of resistant cells in cultures from treated bacteria (tables 1 and 2) as due merely to variations in the growth rates of the mutant clones, these being present from the beginning as actively dividing entities, then mutant clones in which $g=9$ should in these tests give values for $n_1/m_1 \times m_2/n_2$ of approximately 0.0019, those in which $g=6$ values of approximately 0.016, and those in which $g=0$ values of approximately unity. That there are some significant differences in growth rate is indicated in tables 4 and 5, but these are obviously too slight to account for the data presented in the previous section.

Thus, the histograms in figure 2 may be said to represent with a fair degree of accuracy the times of appearance of induced mutants as actively dividing mutant clones. These histograms provide information which is quite parallel to that of DEMEREC (1946) on phenotypic expression. And, combining the evidence from both sources it can be said that in the early stages of the growth of irradiated bacteria most of the induced mutations are neither phenotypically

detectable nor are they present as actively dividing mutant clones which will later develop phenotypic expression (interpretation C of figure 1).

However, whether they are present in a non-dividing state (interpretation D) or whether the treatment has merely acted to raise the spontaneous rate of mutation (interpretations A and B) can be determined only from a study of the progeny of single treated cells.

EVIDENCE FROM THE PROPORTIONS OF RESISTANT BACTERIA IN THE PROGENY OF SINGLE TREATED INDIVIDUALS

To discriminate between the remaining interpretations (A, B, and D of figure 1) it is necessary to determine whether or not the descendents of single treated bacteria may include mixtures of resistant and susceptible cells. Because of the possibility of more than one nucleus in the bacteria any data from mixed clones would have to be considered statistically, but observations of a relatively small number of wholly resistant clones would constitute critical evidence.

To secure cultures from single treated bacteria, colonies were first obtained by growing irradiated suspensions on agar. Broth cultures were then inoculated with single whole colonies picked from the plate by means of a loop, and were incubated until they had reached saturation. (The possibility of a colony having arisen from more than one treated bacterium will be considered later.) The approximate number of resistant cells in each culture was then determined by spreading a loopful (approximately 0.005 cc) of each on agar previously spread with phage *T1*, and incubating. Where the number of surviving colonies indicated a high proportion of resistant bacteria (that is, more than one per 2^{10}), careful tests were made to determine more precisely the numbers of resistant and susceptible cells.

The most probable number of cultures required to obtain one induced mutant clone may be estimated from table 1. For the one minute treatment it appears that approximately 600 would be needed. In practice 3,480 were grown from this material and two containing induced mutant clones were obtained (table 6).

TABLE 6

Proportions of induced mutants (B/r/1) in cultures from single treated bacteria.

EXPERIMENT	A	B
No. of test cultures	1,800	1,680
Vol. of cult., cc	2	2
Bact. in inoc. (n_1)	1	1
Av. end no. bact. (n_2)	7.2×10^8	6.8×10^8
No. of cult. with $g<10.5$	1	1
Cultures with $g<10.5$		
No. of estimates of m_2/n_2	8	8
Av. value of m_2/n_2	$1.04\pm.03$	$0.97\pm.19$
Value of g	0.0	0.0

It was found that these contained resistant bacteria only, and tests were made to ensure that there had been no contamination. First, the bacteria were tested against phage *T2* and found to be susceptible. This identified them as *E. coli* and rendered the possibility of bacterial contamination remote. Second, the cultures were tested for the presence of phage and a negative result indicated that the pure mutant clone had not arisen as the result of accidental contamination with *T1* and subsequent selection.

Further, these two cultures cannot have been derived from phenotypically resistant bacteria present prior to growth ("zero-point mutants") since no such resistant cells were found in samples containing 1.6×10^8 treated bacteria.

Also, it is extremely unlikely that they arose from cells which in cultures from large inocula would have given rise to actively dividing mutant clones at the beginning of growth since only four such clones are shown in table 1, out of a total of 46. From this, the chance that both are of such origin is $(4/46)^2$ which is equal to 0.008.

Thus, mixtures of mutant and non-mutant individuals among the descendents of a single treated bacterium are not characteristic of the "delayed effect." This fact rules out interpretations A and B of figure 1 which assume an induced gene instability.

It now appears that the mechanism outlined under D, in which a delay in the onset of division is combined with a delay in phenotypic expression, must be operative in causing the observed delay in appearance of induced mutations. It is of course conceivable that in addition there are individual instances of induced gene instability, but it would be difficult to establish their existence, and at the present time there is no reason to assume such a dual interpretation.

In the next section evidence concerning the assumed variable delay in onset of division will be conidered.

DELAYED ONSET OF DIVISION IN IRRADIATED CELLS

In the previous section it was shown that to explain the "delayed effect" a delay in the onset of division in the induced mutants such that the bulk of them do not start to divide until the population has increased appreciably must be assumed.

This assumption involves two possible concepts. Either (a) the delay in the onset of division is associated with the presence of the mutation which we are considering or (b) there is a variability in the time of onset of division, not in any way associated with resistance to *T1*, such that the majority of the treated bacteria do not start to divide until the population has risen to many times its original number. The former of these two concepts would seem unlikely on *a priori* grounds, but it remains for the latter to be demonstrated.

Under normal conditions, bacteria from a saturated culture of *E. coli* strain *B/r* inoculated into fresh broth medium and incubated, do not divide until after an interval of approximately seventy minutes (at 37°C). Following this "lag period" the population doubles every 19 to 20 minutes until the saturation density is approached. As a result of irradiation, this lag period is considerably increased, usually by from one to two hours depending upon the dose, but once

divisions have begun (or at least very shortly thereafter) the population rises exponentially at the normal rate (DEMEREC 1946). This, of course, does not necessarily mean that division starts in all of the treated individuals at the same time, and even with wide differences in the time of onset the population increase would become approximately exponential as soon as the actively dividing individuals arising from the early divisions appreciably outnumbered those which had not yet started to divide. For this reason, evidence as to the existence of a cell to cell variation in the onset of division in treated bacteria cannot be obtained from the growth curves of liquid cultures.

In order to obtain a clearer picture of the times at which irradiated bacteria enter division, suitable dilutions of an irradiated suspension (1,000 ergs×millimeters^{-2}; survival = 24.1 percent) were spread on agar and incubated for vary-

TABLE 7

Time of onset of divisions in irradiated B/r, and numbers of induced mutations which become phenotypically detectable. (Dose = 1,000 ergs×millimeters^{-2}; survival = 24.1 percent.)

INCUB. (MIN.)	NUMBERS OF MICROCOLONIES									VIABLE CELLS (EST.*)	INDUCED MUTATIONS (/10^8)†
	CELL GENERATIONS										
	0	1	2	3	4	5	6	7	8		
0	1,000	—	—	—	—	—	—	—	—	240	0
60	1,000	—	—	—	—	—	—	—	—	240	20
80	999	1	—	—	—	—	—	—	—	241	12
100	979	21	—	—	—	—	—	—	—	261	9
120	968	28	4	—	—	—	—	—	—	280	12
140	970	18	8	4	—	—	—	—	—	310	20
160	950	35	6	8	1	—	—	—	—	356	120
180	954	40	0	2	4	—	—	—	—	372	225
200	925	48	7	5	4	9	2	—	—	796	517
210	807	126	35	15	1	6	10	—	—	1,408	795
240	803	104	42	26	3	4	13	4	1	2,404	977

* As no allowance is made for the inviable cells which have undergone a single division, the early figures will represent a slight overestimate. This bias becomes negligible in the later figures.

† Averages based on four plates each; inoculum 2.4×10^7 bacteria; using the method of DEMEREC (1946).

ing periods of time. At the end of incubation the plates were chilled to stop all divisions and were examined under the high power dry objective. From each plate the proportions of bacteria which had undergone 0, 1, 2, 3, 4, etc. divisions were determined. Ten different periods of incubation, differing by 20 minute intervals, were used; there were four replicate plates, and a thousand microcolonies were scored for each of the incubation periods. The results of this experiment are presented in table 7.

Since the intervals were arranged to coincide with the division period of the bacterium, it is possible from the table to determine what proportion of the viable cells enter the first division at a particular time, and what proportion

continue to divide after the first division. It will be seen that well over half of the bacteria which pass through one division do not divide further, but that practically all which pass through the second division go on dividing indefinitely. Thus, only those which pass through two divisions contribute to the effect which we are considering. Of these, it will be seen that only a small proportion take part in division when it first commences, that more than half are delayed until after the first have passed through their fifth division, and that a very appreciable proportion do not divide until after some have passed through the eighth division. Because of the difficulty of estimating the numbers of bacteria in very large microcolonies the observations could not be extended beyond this point.

This picture is qualitatively what is required to explain the "delayed effect" without assuming any correlation between the mutation which is being studied and the delay in onset of division. It does not, of course, eliminate all possibility of such a correlation, but it does show that it is not the major factor.

Thus the "delayed effect" of irradiation is due to a delay of one or more cell divisions between mutation and phenotypic expression (already observed in the case of spontaneous mutations), plus a variability in the time at which irradiated bacteria start to divide.

EXPRESSION OF DELAYED EFFECT

During the present study, two main conclusions have been reached regarding the "delayed effect" of irradiation: (1) that the mutations are present in the irradiated population prior to growth but fail to produce any phenotypic change until one or more cell divisions have taken place in the mutant bacteria, and (2) that many of the mutations occur in individuals which will not start to divide until the treated population has increased considerably.

A delay of two or three cell divisions between spontaneous mutation and phenotypic expression has already been demonstrated (NEWCOMBE 1948) and the simplest interpretation in the case of the induced mutations would be that the delay is of similar origin. There has been one obstacle to a common interpretation namely that induced mutations appeared to take longer to achieve phenotypic expression. Concerning this it has been shown in the present work that the increased time need not necessarily be due to a greater number of cell divisions before induced mutations are expressed, but may be the result of a variability in the time of onset of division of the treated bacteria such that many individuals fail to divide until early growth has produced an appreciable population rise. There remains then little reason to assume that the delayed phenotypic expression in spontaneous and induced mutations is due to different causal factors.

MECHANISM OF DELAYED EFFECT

The delay in phenotypic expression may be interpreted in terms of (a) genetic systems, or (b) enzyme systems (see DEMEREC 1946; DEMEREC and LATARJET 1946).

Genetic interpretations would assume that the effect of the mutated unit is

obscured by the presence of one or more normal homologous units and that expression occurs when individuals containing mutated units only are produced through division and segregation. The reduplicated structures might be:

(1) gene lamellae
(2) cytoplasmic genes
(3) chromosomes
(4) nuclei.

That the delay may be due to the presence of a number of gene lamellae or of cytoplasmic genes is rendered unlikely by the finding of resistant clones from single treated bacteria which are unmixed with susceptible cells. It is still conceivable however, that duplicate sets of chromosomes or duplicate nuclei are present at the time of treatment and that they have been rendered inviable through lethal mutation or structural change.

One of these possibilities, that of diploidy or polyloidy, may be eliminated since spontaneous mutants would be obscured for an indefinite period. The possible presence of more than one nucleus however deserves serious consideration.

There is no cytological evidence of the number of nuclei in resting cells, the stage at which our material was irradiated. However, ROBINOW (1945) and BOIVIN (1947) have observed that rapidly dividing cells contain either two or four staining bodies arranged in linear order within the cell. There is good reason to believe that these are nuclei. Further, they appear to be included in daughter bacteria at cell division in a regular manner merely by the formation of a wall separating sisters if there are only two, or pairs of sisters if there are four.

If this situation exists in the resting stage it could account for the delayed appearance of induced mutants. Thus, where two nuclei are present one cell division would be required for a mutation to be expressed, and where there are four nuclei two divisions would be required.

However, the delay observed in the case of spontaneous mutations (NEWCOMBE 1948) could not be explained in this manner for the following reason. In this interpretation the number of phenotypically resistant bacteria arising from a mutation would double with each cell generation following the appearance of the first one. The number of phenotypically resistant bacteria arising from a spontaneous mutation however more than doubles during each of the three divisions taking place after the first one has appeared, and at the end of this period there are, not eight, but something like six times this number. Thus the presence of more than one nucleus could not account for the observed delay in the case of spontaneous mutations although it might well produce an additional delay which could not be detected by the methods used. (The possibility of a mode of segregation of four nuclei other than that indicated above has been considered but will not be discussed in detail since one has to assume a highly improbable behavior in order to account for the spontaneous mutation data. Briefly, this would involve an almost regular exchange of place of the two inner nuclei.)

Thus it seems likely that non-genetic mechanisms are involved in the de-

layed expression of spontaneous mutations. And, it is reasonable to assume that induced mutations are likewise subject to a non-genetic delay, whether or not a segregation effect is also present.

Relevant to this is McIlwain's (1946) speculation that enzyme production may be associated with gene reproduction. This speculation is based on an argument indicating that the number of molecules of certain enzymes in a cell is small, probably one or two.

However, before the evidence on delayed phenotypic expression of mutation in bacteria can be related to these general problems it will be necessary to know more of the relationship between mutation, delay, and phenotypic expression, and in particular whether this sequence is or is not a function of the mutagenic agent, the environmental factors at the time of the mutation, the particular gene mutation, or, finally, of the organism in which mutation is studied.

What is clear from the evidence so far obtained, is that the delayed appearance of mutations in bacteria following irradiation does not in any way constitute evidence of a fundamental genetic peculiarity of these organisms. Relevant to this general problem, although not to the specific question of delayed phenotypic expression, are the data on irradiation killing. These will be considered in the next section.

KILLING

Following lethal doses of irradiation, bacteria do not disintegrate as when treated with strong disinfectants; motile forms may remain motile, spores may germinate, and bacterial cells may pass through one or two divisions (Bruynoghe and Mund 1935; unpublished observations of Robinow and Lea reported in Lea 1946 page 325; Luria 1939). The present experiments show that the majority of the "killed" cells of *E. coli* strain *B/r* undergo a single division (table 7). The possible implications of this behavior will now be considered.

Irradiation is known to kill the cells of higher organisms through:

(a) non-genetic mechanisms
(b) lethal mutation
(c) chromosome unbalance following breakage and rearrangement.

Non-genetic mechanisms appear to act in the killing of the radiation susceptible strain *B* of *E. coli*. This is indicated by the fact that the sensitive strain *B* and the resistant mutant *B/r* do not differ in their response to the mutagenic action of irradiation (Demerec and Latarjet 1946). Also, strain *B* is more susceptible than *B/r* to such presumably non-mutagenic chemicals as penicillin and sodium sulphathiozole (Witkin 1947). It may thus be concluded that the bulk of killing in strain *B* is not due to genetic effects. However, in strain *B/r* genetic effects may well be significant.

Lethal mutations have been suggested by Lea (1946) as a possible cause of death, and could conceivably account for the delayed killing of *B/r* (table 7) provided that they, like mutations to phage resistance, have a delayed phenotypic expression. This however is not the only possible interpretation.

Chromosome unbalance has long been considered the major cause of cell death in higher organisms following irradiation. Here the effects, in terms of

chromosome breakage and rearrangement, can be studied in detail at metaphase following treatment, and it can be seen that certain of the daughter cells will lack portions of the chromosome material and will contain replicates of other portions.

The expression of this mechanism of killing is complicated in diploid tissues by the presence of a duplicate set of chromosomes which enable the cells to survive considerable loss and reduplication. In haploid cells however the effect is more clear cut. Here it can be shown that irradiation and chromosome breakage has little effect on cell behavior until division occurs, but that after it has taken place normal function ceases. For example in Tradescantia 800 r given after pollen grain division has no detectable effect upon pollen germination, but if given prior to this division germination is inhibited in practically all of the treated grains (NEWCOMBE 1942).

That a chromosomal mechanism has not been suggested earlier for radiation induced killing of bacteria has in all probability been due to the absence in the past of any indication of the existence of bacterial chromosomes. But, in view of the cytological evidence of ROBINOW (1945) and BOIVIN (1947), and the recent genetic evidence of LEDERBERG (1947), this interpretation has become increasingly plausible.

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This investigation was originally contemplated while the senior author was working under DR. M. DEMEREC, but was postponed in favor of a study of spontaneous mutation. The experimental approach is quite different from that originally considered, but much is owed to the early discussions with DR. DEMEREC concerning possible interpretations of the data available at that time.

CONCLUSIONS

Ultra-violet induced mutations in the bacterium *E. coli* strain *B/r*, resulting in resistance to the bacteriophage *T1*, have been studied. It was known that the majority of these mutations do not appear until after the treated population is permitted to grow, some being delayed by as much as twelve generations (or a 2^{12} times increase) of the treated population (DEMEREC, 1946). The present study has yielded the following information regarding the underlying causes of this phenomenon:

(1) The delayed appearance of induced mutations is due (a) to the necessity for one or more cell divisions to take place before mutation can be expressed phenotypically, and (b) to a failure of many of the induced mutant cells to start dividing until the population has increased considerably.

(2) This delay in phenotypic expression appears to be similar to that previously demonstrated in the case of spontaneous mutations (NEWCOMBE, 1948) and it therefore seems probable that it is due to a non-genetic (such as enzyme) mechanism.

(3) There is a wide variation in the time at which individual treated cells start to divide. This accounts for the failure of many of the induced mutants

to commence division until the population has risen considerably, without assuming that the presence of the mutation is responsible for the delay.

Thus delayed appearance of radiation-induced mutants does not indicate any fundamental difference between the genic materials of bacteria and those of higher organisms. Also these experiments indicate a relationship between mutation and expression which would be difficult to demonstrate in multicellular tissues.

Relevant to the question of the genetic similarity of bacteria and higher organisms, it has been found that the majority of the *B/r* individuals "killed" by the ultra-violet irradiation are capable of a single cell division. This behavior is similar to that of the cells of higher organisms and the possibility of a chromosomal mechanism is suggested.

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LINKAGE STUDIES WITH BIOCHEMICAL MUTANTS OF NEUROSPORA CRASSA†*

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IN 1946 a tentative chromosome map of *Neurospora crassa* was published (BEADLE) showing members, among the biochemical mutants obtained at the Stanford laboratory, of the five linkage groups then known. The data have not been published since they were considered not sufficiently extensive, in most cases, to establish relative positions of the various genes within the linkage groups. Since that time, however, a number of requests from other laboratories have indicated that these data may be useful to other workers. They are presented here, along with more recent data.

EXPERIMENTAL RESULTS

The crosses are listed in five tables with the types of segregation obtained tabulated for each cross. Types of segregation are numbered at the head of the table and the number of each type observed is tabulated under the appropriate type-number. The various arrangements of spore pairs possible for each type of segregation are not considered. Tables 1, 2 and 3 contain crosses showing segregation of sex and one mutant, sex and two mutants, and two mutants respectively. Table 4 gives the numbers of first and second division segregations from crosses in which segregation of only one mutant was observed. Table 5 shows results from crosses between two mutants, usually with the same growth requirement, which could not be, or were not, distinguished from one another. Centromere distances cannot, of course, be obtained from these data, but cases of linkage or possible allelism are demonstrated. The crosses are listed numerically by the isolation numbers of the mutants. When two mutants are involved the smaller number is placed first and the cross listed under that number. The following abbreviations for requirements and descriptions have been used:

ad	adenineless	*phen*	phenylalanineless
ad-p	purple adenineless	*prol*	prolineless
arg	arginineless	*pyr*	pyrimidineless
chol	cholineless	*pyro*	pyridoxinless
hist	histidineless	*pyro-p*	pyridoxinless-pH sensitive
inos	inositolless	*ribo*	riboflavinless
isol-val	isoleucine-valineless	*ser*	serineless

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TABLE 1

Crosses showing segregation of sex and one mutant

	TYPES OF SEGREGATION							
	Xa × *+A*				OR	*XA* × *+a*			
1	*Xa*	*Xa*	*+A*	*+A*		*XA*	*XA*	*+a*	*+a*
2	*XA*	*XA*	*+a*	*+a*		*Xa*	*Xa*	*+A*	*+A*
3	*Xa*	*+a*	*XA*	*+A*		*Xa*	*+a*	*XA*	*+A*
4	*Xa*	*XA*	*+a*	*+A*		*Xa*	*XA*	*+a*	*+A*
5	*Xa*	*+A*	*Xa*	*+A*		*XA*	*+a*	*XA*	*+a*
6	*Xa*	*+A*	*XA*	*+a*		*Xa*	*+A*	*XA*	*+a*
7	*XA*	*+a*	*XA*	*+a*		*Xa*	*+A*	*Xa*	*+A*

STRAINS					TYPES OF SEGREGATION							TOTAL	NO. ISOLATED
X × *+*					1	2	3	4	5	6	7		
263	*A pyr*	×	Abbott 12	*a* Wild	11	7	2	3				23	24
*299	*a pyro-p*	×	nonconidial	*A* vis	5	6	2	7		1		21	22
*1090	*a thi*	×	nonconidial	*A* "			6	3		4		13	25
1298	*a pyr*	×	1	*A* Wild	7	7	5					19	20
9185	*A thi*	×	Abbott 12	*a* "	7	1	9	1		1	1	20	20
9460	*a* vis	×	1	*A* "	8							8	10
15300	*A* "	×	25	*a* "	24	3	23	3		1		54	58
15300	*a* "	×	33458	*A* vis	12	2	4	2				20	23
20705	*a ad*	×	Abbott 4	*A* Wild	6	3	13	2				24	24
27663	*a* "	×	Abbott 4	*A* "	3	9	4		1		1	18	23
28610	*a* "	×	Abbott 4	*A* "	8	13	3					24	24
28815	*a* "	×	Abbott 4	*A* "	4	1	10	1			2	18	18
32213	*A un-t*	×	Em 5297	*a* "	5		9		1	2	1	18	20
34508	*A* vis	×	25	*a* "	7	11	17	1				36	40
34542	*A chol*	×	25	*a* "	4	5	20	1	1	1	1	33	34
34547	*A nitr*	×	Chilton	*a* "	18		19	1	1		1	40	43
34547	*a* "	×	Em 5256	*A* "	16		7		1		1	25	30
35402	*a suc*	×	Abbott 4	*A* "	17			1				18	20
35405	*a pyro*	×	1	*A* "	8	4	5	1				18	20
35810	*a isol-val*	×	Abbott 4	*A* "	5	10	3					18	20
37401	*a inos*	×	Abbott 4	*A* "	8	14	20				1	43	45
37602	*a suc*	×	Em 5256	*A* "	12	1	1	5				19	20
38502	*A pyr*	×	Em 5297	*a* "	3	4	9	1	1	1	1	19	20
39106	*A pyro-p*	×	Chilton	*a* "	3	4		3				10	10
39303	*a nic*	×	Abbott 4	*A* "	19			1				20	20
39311	*a suc*	×	Abbott 4	*A* "	16				2			18	20
39401	*a nic-tryp*	×	Abbott 4	*A* "	7	7	4	1				19	20
39404	*a suc*	×	Em 5256	*A* "	15		1	3	1			20	20
40008	*A tryp*	×	Abbott 12	*a* "	4	6	4					14	20
43002	*A nic*	×	25	*a* "	19							19	20
44008	*a tryp*	×	1	*A* "	8	6	3		1			18	20
44101	*a pyr*	×	Abbott 4	*A* "	5	6	1	6				18	18
44204	*a pyro-p*	×	1	*A* "	5	4		2				11	11
44206	*A ad-t*	×	25	*a* "	10	9		1				20	20

TABLE 1—(*continued*)

STRAINS					TYPES OF SEGREGATION							TOTAL	NO. ISOLATED
	X	×	+		1	2	3	4	5	6	7		
44302 *A pyr*	×	25	*a*	"	7	5	2	4				18	20
44409 *a un-t*	×	Abbott 4	*A*	"	14		4	1			1	20	20
44411 *a ad*	×	1	*A*	Wild	1	2	12			3	1	19	20
44504 *A pyr*	×	Abbott 12	*a*	"	8	6	2	5	1			22	24
44601 *A un*	×	25	*a*	"	8	10		3				21	24
44602 *A pyro-p*	×	Chilton	*a*	"	4	7	4	1				16	20
45004 *A ad*	×	Abbott 12	*a*	"	2	5	7	5		1		20	20
45208 *a un-t*	×	Abbott 4	*A*	"	1		6		1	1		9	10
45304 *A tryp*	×	25	*a*	"	11	16	4		1		1	33	40
45502 *A pyr*	×	Abbott 12	*a*	"	10	7		5				22	22
45612 *A un*	×	Abbott 12	*a*	"	4	10		4		1		19	20
46006 *a un-t*	×	Abbott 4	*A*	"	15			3				18	20
46403 *a un*	×	1	*A*	"	11		1		2		1	15	20
46407 *A prol*	×	25	*a*	"	6		6	2	1			15	20
46705 *A ad*	×	Abbott 12	*a*	"	4	2	7	2	1			16	20
46904 *A pyro-p*	×	Chilton	*a*	"	3	4	1	2				10	10
47305 *A un-t*	×	25	*a*	"	3	4	6	1	2	1	1	18	20
47807 *a suc*	×	1	*A*	"	16			1	2	1		20	20
47904 *a chol*	×	Abbott 4	*A*	"	3	1	6		1	1	1	13	20
51505 *A prol*	×	Abbott 12	*a*	"	2	5	9					16	20
51602 *a ribo-t*	×	Abbott 4	*A*	"	5	14		1				20	20
55701 *a un-t*	×	1	*A*	"	36				2			38	40
56501 *a thi*	×	1	*A*	"	6		7	1	1			15	20
63904 *a pyr*	×	Abbott 4	*A*	"	2	5	7	4		1	2	21	24
66204 *A un-t*	×	25	*a*	"	8	8						16	20
66210 *A chol*	×	Chilton	*a*	"	1	1	9	2		2		15	20
66702 *a suc*	×	1	*A*	"	6			1				7	8
67602 *a pyr*	×	Abbott 4	*A*	"	6	6	1	4				17	20
67701 *a pyr*	×	1	*A*	"	5	7	1	2			1	16	20
68305 *a pyr*	×	Abbott 4	*A*	"	2	10	7	5				24	24
70004 *A ad-t*	×	25	*a*	"	3	4	4	1				12	20
71104 *a ad*	×	1	*A*	"	16			3	1			20	20
75001 *A tryp*	×	25	*a*	"	9	4	5	1				19	20
83106 *a un-t*	×	Abbott 4	*A*	"	4	8	5	1				18	20
85902 *A thi*	×	Em 5297	*a*	"	11	8		18				37	40
												1392	1548

* *Neurospora sulophila.*

leu	leucineless	*sfo*	sulfonamide-requiring
lys	lysineless	*suc*	succinicless
meth	methionineless	*thi*	thiaminless
nic	nicotinicless	*thr*	threonineless
nic-tryp	nicotinic or tryptophanless	*tryp*	tryptophanless
nitr	unable to use nitrate	*un*	unknown requirement
pab	para aminobenzoicless	*val*	valineless
pan	pantothenicless		

TABLE 2

Crosses showing segregation of sex and two mutants

	TYPES OF SEGREGATION			
	$X+a \times +yA$			
1	$X+a$	$X+a$	$+yA$	$+yA$
2	$X+A$	$X+A$	$+ya$	$+ya$
3	$X+a$	$X+A$	$+ya$	$+yA$
4	Xya	Xya	$++A$	$++A$
5	XyA	XyA	$++a$	$++a$
6	Xya	XyA	$++a$	$++A$
7	$X+a$	Xya	$++A$	$+yA$
8	$X+A$	XyA	$++a$	$+ya$
9	$X+a$	XyA	$++a$	$+yA$
10	$X+A$	Xya	$++A$	$+ya$
11	$X+a$	XyA	$++A$	$+ya$
12	$X+A$	Xya	$++a$	$+yA$
13	$X+a$	$++a$	XyA	$+yA$
14	$X+A$	$++A$	Xya	$+ya$
15	$X+a$	$++A$	Xya	$+yA$
16	$X+A$	$++a$	XyA	$+ya$
17	$X+a$	$++A$	XyA	$+ya$
18	$X+A$	$++a$	Xya	$+yA$
19	$X+a$	$+ya$	$X+A$	$+yA$
20	$X+a$	$+yA$	$X+a$	$+yA$
21	$X+A$	$+ya$	$X+A$	$+ya$
22	$X+a$	$+yA$	$X+A$	$+ya$
23	$X+a$	$+ya$	XyA	$++A$
24	$X+A$	$+yA$	Xya	$++a$
25	$X+a$	$+yA$	Xya	$++A$
26	$X+A$	$+ya$	XyA	$++a$
27	$X+a$	$+yA$	XyA	$++a$
28	$X+A$	$+ya$	Xya	$++A$
29	Xya	$++a$	XyA	$++A$
30	Xya	$++A$	Xya	$++A$
31	XyA	$++a$	XyA	$++a$
32	Xya	$++A$	XyA	$++a$

(*This table continues on facing page*)

The appearance of *-t* after a symbol indicates that the mutant is temperature sensitive. All morphological mutants as well as the albinos are listed as "v s," abbreviating visible.

In tables 1, 2, 3 and 4 asci are included if at least one member of each of three spore pairs germinated and could be tested. In table 5 only asci from which at least one member of each spore pair germinated are included. It should be pointed out that if failure of spores to mature or to germinate is associated with particular genotypes or types of segregation then selection of complete asci for dissection, and after germination, for classification, will lead to errors which are not easily avoided. Crosses which gave few complete asci, as well as those from which germination was poor, were rather frequently encountered in the course of this work, particularly when two mutants having the same growth requirement were involved.

In table 6 an attempt is made to summarize the information pertinent to linkage by listing the mutants in the appropriate linkage group, when this is known. Centromere distance was calculated from all crosses (except those in

TABLE 2—(*continued*)

STRAINS				TYPES OF SEGREGATION									TOTAL	NUMBER ISOLATED
X+	×	*+y*												
517	*A fat*	× 4637	*a* vis	1-9	2-1	5-12	13-1						23	27
1633	*A pab*	× 4637	*a* "	1-2	2-1	5-4	7-2	13-9	14-1	16-1			20	20
1752	*A fat*	× 4637	*a* "	1-3	2-1	13-5	14-3	24-1					13	17
3254	*a ad*	× 4637	*A* "	1-5	3-1	4-1	5-10	7-1					18	20
3416	*a nic*	× 4637	*A* "	1-18	2-2	13-1	18-1	19-1					23	31
3717	*A* vis	× 55701	*a un-t*	1-13	9-3								16	16
4540	*A nic*	× 4637	*a* vis	1-15	3-1	4-1	7-1						18	33
4637	*a* vis	× 5531	*A pan*	1-1	2-1	4-4	7-9	8-1	12-1	13-1	14-1		19	20
4637	*A* "	× 9185	*a thi*	1-1	3-1	4-2	7-2						6	6
4637	*a* "	× 10575	*A tyrp*	2-2	4-4	5-1	7-6	8-1	12-1	29-1			16	18
4637	*a* "	× 14789	*A n²tr*	1-2	4-2	7-8	11-1	14-1	19-1	23-1			16	20
4637	*a* "	× 16117	*A isol-val*	1-5	2-1	3-2	4-6	7-2	8-1	12-1	13-1	14-1		
				24-1									21	22
4637	*A* "	× 21863	*a prol*	1-11	2-3	3-7	4-14	6-2	7-2	13-3	14-2	29-1	45	45
4637	*A* "	× 33026	*a val*	1-7	3-1	4-4	6-1	7-1	13-3	14-2	29-1		20	20
4637	*A* "	× 34486	*a chol*	1-5	4-4	6-1	7-8	9-1	16-1				20	20
9460	*a* "	× 49001	*A pyr*	1-6	4-7	7-2	13-1	14-1					17	20
15300	*A* "	× 38701	*a ad-p*	1-11	3-2	5-1	13-19	15-1	16-1	18-1	23-2		38	46
28815	*A lys*	× 34508	*a* vis	1-6	2-1	3-2	19-8	20-2	24-1				20	23
34508	*a* vis	× 37301	*A pyr*	1-2	3-1	7-3	14-4						10	10
34508	*a* "	× 38620	*A phen*	1-5	4-5	6-1	7-1	13-3	14-1	19-2	20-1	21-1		
				24-1	29-1								22	24
34547	*a* nitr	× 34547–10	*A tryp*	1-2	6-1	7-2	8-1	9-1	12-1	13-7	14-8	16-1		
				17-1	18-1	22-1	23-1	24-2	25-2	29-1			33	40
55701	*a* un-t	× 68902	*A pyr*	1-2	4-9	7-8	15-2	25-1					22	24
													456	521

Note: The number of the segregation type precedes the number of asci of that type. Segregation type 1 represents asci in which sex and both mutant genes segregated in the first division and there was no recombination. Type 2 represents those in which the three genes segregated in the first division but in which *y* and sex and *X* and sex recombined, etc., etc.

table 5) involving a given gene and no corrections were made. All of the genes which appear on the tentative map published earlier (BEADLE 1946) are listed here although in some cases linkage was shown by other workers at the Stanford laboratory, namely BELL, BUSS, DOERMANN, F. HUNGATE, REGNERY and SRB. Their data are not included here, but, when possible, references are given. When relative positions of genes are not indicated by the data presented here the order given is based simply on centromere distance. When numbers of mutants are indented under the number of another mutant with the same requirement this indicates that attempts to demonstrate non-allelism have, so far, been unsuccessful. A small "l" following the description of a mutant means that the mutant has been lost.

In only one case, that of the sex linkage group, do these data give evidence that any of the genes listed are on opposite sides of the centromere. In the sex group there is evidence, although in many cases it is based on rather limited data, that the genes are located in the arm in which they are listed. No crosses have been made between any of the mutants which appear in the same arm as sex. In the other arm, 3416 and 34547 are shown to be farther from the centromere than 4637. The data of M. HUNGATE (1945) indicate that 15300 is ge-

TABLE 3

Crosses showing segregation of two mutants.

	TYPES OF SEGREGATION							
	$X+ \times +y$				$Xy \times ++$			
1	$X+$	$X+$	$+y$	$+y$	Xy	Xy	$++$	$++$
2	Xy	Xy	$++$	$++$	$X+$	$X+$	$+y$	$+y$
3	$X+$	Xy	$++$	$+y$	$X+$	Xy	$++$	$+y$
4	$X+$	$++$	Xy	$+y$	$X+$	$++$	Xy	$+y$
5	$X+$	$+y$	$X+$	$+y$	Xy	$++$	Xy	$++$
6	$X+$	$+y$	$++$	Xy	$X+$	$+y$	$++$	Xy
7	Xy	$++$	Xy	$++$	$X+$	$+y$	$X+$	$+y$

STRAINS				TYPES OF SEGREGATION							TOTAL	NUMBER ISOLATED
$X+ \times +y$ / $Xy \times ++$				or	1	2	3	4	5	6	7		
263	*a pyr*	× 4545	*A lys*		2		7		1			10	10
263	*A* "	× 44204	*a pyro-p*		24				6			30	40
263	*a* "	× 37815	*A pyr-t*		79		7	1	11			98	120
263	*a* "	× 67602	*A pyr*		52		9		5			66	70
1298	*a* "	× 4545	*A lys*				5			3		8	10
1633	*a pab*	× fluffy	*A* vis		1	2	6	4	1	2		16	20
1633	*A* "	× 45304	*a tryp*		2		1	6			1	10	11
3254	*A ad*	× 21863	*a prol*		7	4						11	12
3254	*a* "	× 16117	*A isol-val*		8	9	1	2				20	20
3254	*a* "	× 37401	*A inos*		7	4	8					19	20
3254	*a* "	× 38602	*A phen*		7		4			1		12	12
3254, 38701	*A ad, ad-p*	× Abbott 12	*a* Wild		8	11						19	20
3254	*a ad*	× 44206	*A ad-t*		4	12	4					20	20
3254, 44206	*a ad, ad-t*	× 1	*A* Wild		11	8	1					20	20
3254	*a ad*	× 45502	*A pyr*		10							10	10
3254	*a* "	× 51602	*A ribo-t*		17							17	20
3254	*a* "	× 66204	*A un-t*		14		5					19	20
3254	*a* "	× 70004	*A ad-t*		5	6	9					20	20
3254, 70004	*a ad, ad-t*	× 1	*A* Wild		6		8					14	20
3717	*A* vis	× 67602	*a pyr*		6	5	4	2	1	2		20	20
4545	*A lys*	× 37301	*a* "		2		1	4	2			9	10
4545, 37301	*A lys, pyr*	× Em. 8815-3	*a* Wild		2			4		1	2	9	10
4545	*A lys*	× 37815	*a pyr-t*				2	7	1			10	10
4545	*a* "	× 38502	*A pyr*				3	2	2	3		10	10
4545	*a* "	× 67602	*A pyr*		4			4				8	10
4637, 9185	*a* vis, *thi*	× 1	*A* Wild		6	6	3	2		2	1	20	20
5531	*A pan*	× 10575	*a tryp*		6	3	2	8			1	20	20
5531	*A* "	× 33933	*a lys*		5	9	1	10	3			28	38
5531	*A* "	× 34542	*a chol*		13			1	9			23	24
5531	*A pan*	× 37301	*a pyr*		7			4	4	1		16	18
5531	*A* "	× 37803	*a pyro*		15	1	1	17	13	2		49	50
5531	*A* "	× 37903	*a chol*		5				14			19	20
5531	*A* "	× 44101	*a pyr*		15			12	1			28	30
5531	*a* "	× 44104	*a thr*		9	5		5				19	20
5531	*A* "	× 45502	*a pyr*		27			2				29	30
5531	*A* "	× 47904	*a chol*		3	1	4	4	1	4	2	19	20
5801	*a* vis	× 21863	*A prol*		9		1					10	10
9185, 15300	*A thi*, vis	× Chilton	*a* Wild		2		5		2	3		12	20
9185	*A thi*	× 37803	*a pyro*		5	3	1	7				16	20
9460	*a* vis	× 47102	*A pyr*		4	10	1					15	20
9460	*a* "	× 63902	*A* "		3	8	6			1		18	20
9666	*A meth*	× 51602	*a ribo-t*		5	10		5				20	20
10575	*A tryp*	× 34508	*a* vis			3	13	3	2	2		23	24
15069	*a lys*	× 37301	*A pyr*		2	7	4			1		14	20

TABLE 3—(*continued*)

STRAINS				TYPES OF SEGREGATION							TOTAL	NUMBER ISOLATED
	$X+ \times +y$ or $Xy \times ++$			1	2	3	4	5	6	7		
15300	*A* vis	× 34508	*a* vis	19				13			32	36
15300	*a* "	× 34542	*A chol*	3	4	9	11	2	3	3	35	36
16117	*a isol-val*	× 33757	*A leu*	12	5		4	1			22	22
16117	*A* " "	× 37401	*a inos*	9		7		4			20	20
16117	*A* " "	× 39401	*a nic-tryp*	4	2	16		2	2		26	40
16117	*a* " "	× 44206	*A ad-t*	7	11		2				20	20
16117	*a* " "	× 45502	*A pyr*		7		1				8	20
16117	*a* " "	× 47904	*A chol*	2	1	11	2		1		17	20
16117	*A* " "	× 63904	*a pyr*	12	5	1	3			1	22	30
16117	*a* " "	× 66204	*A un-t*	7	7	1	3		1		19	20
16117	*A* " "	× 68305	*a pyr*	7	5	2	4		2		20	30
16117	*A* " "	× As 7980-5	*a* vis	3	6	4	5		2		20	20
17084	*a thi*	× 37803	*A pyro*	4	9	3					16	20
17084	*a* "	× 44602	*A* "	8	2	7					17	20
20705	*A ad*	× 38709	*a ad-p*	3	4		1				8	10
20705, 38709	*a ad, ad-p*	× Abbott 4	*A* Wild	3	1		13				17	20
21863	*a pro*	× 34508	*A* vis	7	12	18	1	2	1		41	47
21863	*a* "	× 44206	*A ad-t*	20							20	20
21863	*a* "	× 47904	*A chol*	4		8		2	4		18	20
21863	*A* "	× 51602	*a ribo-t*	7	9		1	1			18	20
27663	*A ad*	× 38709	*a ad-p*	4	3		2				9	10
27663, 38709	*a ad, ad-p*	× Abbott 4	A Wild	3	2		15				20	20
27663	*A ad*	× 44206	*a ad-t*	11				3			14	20
27663	*a* "	× 44206	*A* "	55				3			58	60
27663	*a* "	× 70004	*A* "	28				5			33	40
28610, 35203	*a ad, ad-p*	× Abbott 4	*A* Wild	7	3		7	2			19	20
28610	*A* "	× 44206	*a ad-t*	8	2	2	6				18	20
28610, 44206	*A ad, ad-t*	× 25	*a* Wild	9	6	2	3				20	20
28815	*a lys*	× 34508	*A* vis	5		2		4			11	22
33757	*A leu*	× 33933	*a lys*	8	3		4				15	45
33757	*A* "	× 37401	*a inos*	4	3	5	2				14	14
33757	*a* "	× 66204	*A un-t*	5	4	2	5				16	20
33933, 37301	*A lys, pyr*	× Em. 88153	*a* Wild	6	2		1				9	10
33933	*A lys*	× 44101	*a pyr*	8	7						15	20
34508	*a* vis	× 34542	*A chol*	3	5	3	9	1	4	3	28	34
34508	*a* "	× 34547	*A nit*	5		1		4			10	10
34508	*a* "	× 35301	*A ad*	2	3	5	4	2	1		17	20
34508	*A* "	× 37401	*a inos*	1	1	4	8	1	3	2	20	20
34508, 83201	*A* vis, *inos*	× Em 5297	*a* Wild	4	2	23	36	19	22	16	122	179
35203, 35301	*a ad-p, ad*	× Abbott 4	*A* "	11	3	2					16	20
35203, 44206	*a ad-p, ad-t*	× 1	*A* "	4	11	1					16	20
35203	*a ad-p*	× 44411	*A ad*		7	13				1	21	40
35203	*a* "	× 70004	*A ad-t*	5	5	6	1				17	40
35405	*A pyro*	× 37803	*a pyro*	42				5			47	50
35405	*A* "	× 39106	*a pyro-p*	18				2			20	20
35405	*A* "	× 39706	*a* "	17				3			20	20
35405	*A* "	× 44204	*a* "	14		1		5			20	20
35405	*a* "	× 44602	*A* "	13				2			17	20
35405	*A* "	× 44602	*a* "	17				3			20	20
35405	*A* "	× 46904	*a* "	20							20	20
36104	*A meth*	× 51602	*a ribo-t*	2	6		10		1	1	20	20
37301	*A pyr*	× 37401	*a inos*	1	1	6	6	2	2	2	20	20
37301	*a* "	× 37803	*A pyro*	31			1	7			39	40
37301	*A* "	× 37815	*a pyr-t*	18				18			36	40
37301	*a* "	× 37815	*A* "	33				7			40	40
37301	*a* "	× 38602	*A phen*	2	4	2	4	1			13	16
37301	*A* "	× 55701	*a un-t*	3	4	1	1				9	10
37301	*a* "	× 67602	*A pyr*	20				9			29	40
37401	*a inos*	× fluffy	*A* vis	1	2	5	7	1	2	2	20	20
37401	*A inos*	× 83201	*a inos-t*	10				10			20	20

TABLE 3—(*continued*)

STRAINS				TYPES OF SEGREGATION							TOTAL	NUMBER ISOLATED
	$X+ \times +y$ $Xy \times ++$			or	1	2	3	4	5	6	7		
37803	*a pyro*	× As-7980-5	*A* vis		4	2	3	6				15	20
37803	*A* "	× 39401	*a nic-tryp*		7	9	11	5				32	40
37803	*A* "	× 50005	*a thi*		6		7	3	4			20	20
37803	*a* "	× 51602	*A ribo-t*		4	5						9	20
37815, 38502	*A pyr-t, pyr*	× Em 8815-3	*a* Wild		4	1	5					10	10
37815	*A pyr-t*	× 44101	*a pyr*		15				2			17	20
37815	*a* "	× 67602	*A* "		26				5			31	41
37903	*a chol*	× 45502	*A* "		12			1	1	1		15	20
38502	*A pyr*	× 55701	*a un-t*		2	1	3	2		1		9	10
38502	*A* "	× 65001	*a nic-tryp*				3	6			1	10	10
39401	*a nic-tryp*	× 44101	*A pyr*		4	3		6				13	30
39401	*a* "	× 44206	*A ad-t*		2	2		9				13	18
39401	*a* "	× 51602	*A ribo-t*		6	5		3			1	15	15
44101	*A pyr*	× 44204	*a pyro-p*		14				5			19	20
44101	*a* "	× 66204	*A un-t*		1	5				2		8	20
44206, 44411	*A ad-t, ad*	× 25	*a* Wild		2		8	1	1			12	20
44206	*A ad-t*	× 45502	*a pyr*		4	7		9				20	20
44206	*A* "	× 70004	*a ad-t*		8		1		7			16	20
44206, 70004	*a ad-t, ad-t*	× 1	*A* Wild		21	1	2		4			28	40
44206	*A ad-t*	× 71104	*a ad*		9	9	1					19	20
44206, 71104	*A ad-t, ad*	× Abbott 12	*a* Wild		4	6	3	3				16	20
44411	*A ad*	× 70004	*a ad-t*		3		4	4	3		2	16	20
44411, 70004	*a ad, ad-t*	× 1	*A* Wild			1	6	8	1		1	17	20
47904	*a chol*	× 66204	*A un-t*		6		1	1				8	10
												2655	3164

netically different from 4637 and 34508, but the relative positions of these two loci are not known. DOERMANN's (1946) work has shown 4545 to be more distant than 15300, while the work of BUSS (1945), DEROMEDI (whose data appear here) and SRB (1947) shows 35809, 38701 and 27947 to be nearer the centromere than 15300. As can be seen from table 5, 18558 shows linkage to 17084 and 56501, but there are no crosses from which its centromere distance can be calculated. In Group B 3254 is nearer the centromere than 66204 and 38602. There are no crosses which show other relative locations. In Group C BELL, F. HUNGATE and REGNERY have shown 5801, 33757, 10575 and 9185 to be located in the order given, while F. HUNGATE has shown that 605 is located between 5801 and 21863. The data given here show 5801 nearer than 21863, and 44206 nearer than 70004. In Group D the order is established by the data with three exceptions. The relative positions of 263 and the pyridoxin-less genes are not shown, nor is the position of 38502 relative to 5531, 9666 and 34486. BUSS (1944) has shown 9666 to be more distant than 37301 but its position relative to 34486 is not known. In Group E the data here show the order of 16117 and 37401 while BUSS (1944) has shown the position of 36104 relative to these two genes. Linkage of 33933 to 37401 was shown by CALHOUN (1945) in a cross from which spores were isolated at random. On the map previously published (BEADLE 1946) 33933 was placed on the opposite side of the centromere on the basis of the distance, obtained from this cross, between

the two genes, and the centromere distance of 37401. Genes for which no linkage has been observed are listed also, since some idea of their location relative to the centromere can be obtained from these data.

It will be seen that 45502 shows linkage to Group B as well as to Group D. This is explained by the fact, reported by McCLINTOCK (1945) that this strain carries a translocation. McCLINTOCK (1945) reported a translocation in strain 4637 also, and SINGLETON (1948) has studied these two, among others, both

TABLE 4

Crosses showing segregation of one mutant.

STRAINS						SEGREGATION		TOTAL	NUMBER ISOLATED
MUTANT		×	WILD, OR OTHER STRAIN			1ST DIVISION	2ND DIVISION		
C 84	*A hist*	×	Em 8815-3	*a*	Wild	7	3	10	10
1633	*A pab*	×	Abbott 12	*a*	"	8	27	35	41
3254	*a ad*	×	Abbott 4	*A*	"	18		18	20
3254	*A* "	×	Chilton	*a*	"	26		26	30
4637	*a* vis	×	Abbott 4	*A*	"	43	1	44	50
5531	*A pan*	×	peach	*a*	vis	9	8	17	20
5801	*A* vis	×	Abbott 12	*a*	wild	29	1	30	31
9185	*A thi*	×	Em 5297	*a*	Wild	104	28	132	180
9185	*A* "	×	Abbott 12	*a*	"	20	21	41	75
17084	*a* "	×	Em 5256	*A*	"	16	1	17	20
21863	*a prol*	×	tuft	*A*	vis	17	3	20	24
34556	*A pan*	×	Abbott 12	*a*	Wild	9	6	15	20
37401	*A inos*	×	peach	*a*	vis	12	8	20	20
37803	*A pyro*	×	Abbott 12	*a*	Wild	15	4	19	20
37815	*a pyr-1*	×	Em 5256	*A*	"	9	4	13	20
37903	*a chol*	×	Abbott 4	*A*	"	25	5	30	40
38502	*a pyr*	×	1	*A*	"	11	25	36	40
39706	*A pyro-p*	×	Chilton	*a*	"	6	1	7	20
39709	*A isol-val*	×	Abbott 12	*a*	"	9	3	12	20
44104	*A thr*	×	Abbott 12	*a*	"	8	8	16	18
44206	*a ad-1*	×	peach	*A*	vis	35	4	39	40
44206	*A* "	×	Abbott 12	*a*	Wild	10		10	10
45208	*a un-1*	×	Abbott 4	*A*	"	6	3	9	20
46807	*A isol-val*	×	Abbott 12	*a*	"	11	3	14	20
47101	*a isol-val*	×	1	*A*	"	18	2	20	20
48504	*A ad*	×	25	*a*	"	12	8	20	20
55701	*a un-1*	×	1	*A*	"	119	12	131	180
66702	*a suc*	×	1	*A*	"	11		11	12
71103	*A un*	×	Em 5297	*a*	"	11	7	18	20
								830	1061

genetically and cytologically. The genetic behavior of 17084 and 27663 suggests the presence of chromosomal aberrations. The former behaves as an allele of 56501 but the two centromere distances differ widely. Also, both

TABLE 5

Crosses involving two mutants not distinguished from each other

Types of asci				
1	m	m	m	m
2	m	m	m	+
3	m	m	+	+

STRAINS				TYPES OF ASCI			TOTAL	NUMBER ISOLATED
				1	2	3		
263	*a pyr*	× 1298	*A pyr*	40	1		41	50
263, 1298	*A pyr, pyr*	× Abbott 12	*a* Wild		6	25	31	40
263	*a pyr*	× 37301	*A pyr*	32	5		37	40
263, 37301	*A pyr, pyr*	× Abbott 12	*a* Wild		8	25	33	40
263, 37815	*a pyr, pyr-1*	× Abbott 4	*A* "		2	16	18	40
263	*a pyr*	× 38502	*A pyr*	6	11		17	20
263, 38502	*a pyr, pyr*	× Abbott 4	*A* Wild	1	7	5	13	30
263	*a pyr*	× 44101	*A pyr*	16			16	20
263	*a* "	× 44302	*A* "	33	2		35	40
263, 44302	*A pyr, pyr*	× 25	*a* Wild			15	15	20
263	*a* " "	× 45502	*A pyr*	13			13	20
263, 67602	*A* " "	× Abbott 12	*a* Wild		2	26	28	40
263	*A pyr*	× 67701	*a pyr*	50			50	60
263	*a* "	× 67701	*A* "	31	1		32	40
1298	*a* "	× 38502	*A* "	7	18	1	26	30
3254	*a ad*	× 20705	*A ad*	3	5	9	17	20
3254	*a ad*	× 27663	*A* "	5	3	7	15	20
3254	*a* "	× 28610	*A* "	2	8	5	15	20
3254	*a* "	× 35301	*A* "	5	11		16	20
3254	*a* "	× 44206	*A ad-1*	8	1	4	13	20
3254	*A* "	× 44411	*a ad*	4	5	1	10	20
3254	*a* "	× 44411	*A* "	4	14	1	19	20
3254	*A* "	× 44415	*a ad-1*	6	3	8	17	20
3254	*A* "	× 71104	*a ad*	11	1	8	20	20
3416	*A nic*	× 39303	*a nic*	4	7	2	13	40
4540	*a* "	× 39113	*A* "	1	3	1	5	20
9185	*A thi*	× 17084	*a thi*	7	3	3	13	20
9185	*a* "	× 18558	*A* "	6	7	5	18	22
9185	*A* "	× 50005	*a* "	5	18	6	29	40
9185	*A* "	× 56501	*a* "	3	18	3	24	40
9185	*A* "	× 85902	*a* "	13	3	1	17	20
14789	*A nitr.*	× 34547	*a nitr.*	3	11	6	20	20
14789, 34547	*a* "	× Em 5256	*A* Wild	10	19		29	40
16117	*A isol-val*	× 35810	*A isol-val*	20			20	20
16117	*A* " "	× 39709	*a* " "	37	1		38	40
16117	*a* " "	× 39709	*A* " "	9		1	10	20
16117	*a* " "	× 46807	*A* " "	10	1		11	20
17084	*a thi*	× 18558	*A thi*	17			17	20

TABLE 5—(*continued*)

STRAINS				TYPES OF ASCI			TOTAL	NUMBER ISOLATED
				1	2	3		
17084	*A* "	× 50005	*a* "	6	20	3	29	30
17084	*A* "	× 56501	*a* "	17			17	20
18558	*A* "	× 50005	*a* "	5	10	5	20	20
18558	*A* "	× 56501	*a* "	1	9		10	20
20705	*A ad*	× 28610	*a ad*	3	5	7	15	20
20705	*a ad-*	× 35301	*A ad*	1	3	1	5	20
20705	*a* "	× 44411	*A* "	6	12	3	21	40
20705	*A* "	× 46705	*a* "	3	5	7	15	20
21863	*a prol*	× 46407	*A prol*	6	3	6	15	20
21863, 46407	*A prol, prol*	× Abbott 12	*a* Wild	1	5		6	20
27663	*A ad*	× 28610	*a ad*	2	3	3	8	20
27663	*a* "	× 35301	*A* "		4		4	10
27663	*A* "	× 44411	*a* "	4	7	2	13	20
27663	*A* "	× 71104	*a* "	4	6	5	15	20
27663, 71104	*A ad, ad*	× Abbott 12	*a* Wild	2	13		15	20
28610	*A ad*	× 44411	*a ad*	4	8	2	14	20
28610	*A* "	× 71104	*a* "	4	1	7	12	20
34486	*A chol*	× 34542	*a chol*	18			18	24
34486	*a* "	× 47904	*A* "	3	11	1	15	30
34486	*a* "	× 66210	*A* "	3	4	4	11	20
34542	*A* "	× 37903	*a* "	18			18	20
34542	*A* "	× 47904	*a* "	3	15	2	20	20
35301	*a ad*	× 44411	*A ad*	3	7	3	13	20
35405	*A pyro*	× 44204	*a pyro-p*	25			25	40
37301	*A pyr*	× 38502	*a pyr*	7	11		18	40
37401	*A inos*	× 83201	*a inos-t*	19			19	20
37815	*a pyr-t*	× 38502	*A pyr*	12	5	1	18	20
37815, 38502	*A pyr-t, pyr*	× Chilton	*a* Wild	1	8	1	10	10
37815	*a pyr-t*	× 67602	*A pyr*	73			73	100
38502	*a pyr*	× 67602	*A pyr*	15	7		22	40
38502, 67602	*a pyr, pyr*	× 1	*A* Wild		4	2	6	10
39113	*A nic*	× 39401	*a nic-tryp*			5	5	9
39303	*a* "	× 39401	*A* "	1	5	15	21	40
39401	*a nic-tryp*	× 43002	*A nic*	10	14	13	37	50
44101	*a pyr*	× 67602	*A pyr*	9			9	20
44206	*A ad-t*	× 66204	*a un-t*	7	4	6	17	20
44409	*A un-t*	× 45208	*a* "	4	15	1	20	20
44409	*a* "	× 46006	*A* "	20			20	20
44411	*A ad*	× 71104	*a ad*	4	10	4	18	20
46407	*a prol*	× 51505	*A prol*	15			15	20
47904	*a chol*	× 66210	*A chol*	7			7	20
50005	*a thi*	× 56501	*A thi*	7	13	8	28	30
55701, 66204	*a un-t, un-t*	× 1	*A* Wild	7	4	4	15	20
66204	*A un-t*	× 83106	*a un-t*	10	7	2	19	20
70004	*A ad-t*	× 71104	*a ad*	4	3	2	8	20
							1572	2195

TABLE 6

Linkage group and centromere distance.

GROUP	MUTANT		NUMBER OF CROSSES	NUMBER OF ASCI	CENTROMERE DISTANCE	LINKAGE FIRST DEMONSTRATED BY
A	sex		88	1811	5.80	
	39311	*suc*	1	18	5.6	
	55701	*un-t*	5	216	4.9	
	71104	*ad*	3	55	4.5	
	15069	*lys*	1	14	3.6	DOERMANN (1946)
	4540	*nic*	1	18	0	
	39303	"	1	20	0	
	43002	"	1	19	0	
	Centromere					
	35203	*ad-p*	5	89	2.1	
	38701	"	2	57	1.8	
	38709	"	4	54	0	
	9460	vis (*1*)	4	55	2.6	
	35402	*suc*	1	18	0	
	37602	"	1	19	2.8	
	39404	"	1	19	2.5	
	47807	"	1	19	7.5	
	66702	"	1	19	0	
	3717	vis	2	36	6.9	
	44409	*un-t*	1	20	10	
	46006	"	1	18	0	
	46403	*un*	1	19	16	
	17084	*thi*	3	50	1.0	
	56501	"	1	15	27	
	15300	vis	6	191	24.6	
	4637	"	16	342	4.97	
	34508	"	13	392	29.84	
	3416	*nic*	1	23	6.5	
	39113	"				
	34547	*nitr*	4	108	27.7	
	4545	*lys*	6	64	37.5	DOERMANN (1946)
	28815	"	2	31	24.2	
	18558	*thi*				
	27947	*arg*				SRB (1947)
	35809	*meth*				BUSS (1944)
B	3254	*ad*	15	266	0.56	
	45502	*pyr*	6	104	0.96	
	51602	*ribo-t*	7	111	1.7	
	66204	*un-t*	6	86	6.97	
	38602	*phen* (*1*)	3	47	4.8	
C	85902	*thi*	1	37	0	
	5801	vis	2	40	1.2	REGNERY (1947)
	21863	*prol*	8	183	5.19	
	44206	*ad-t*	19	399	5.64	
	44415	"				

TABLE 6—(*continued*)

GROUP	MUTANT		NUMBER OF CROSSES	NUMBER OF ASCI	CENTRO-MERE DISTANCE	LINKAGE FIRST DEMONSTRATED BY
	33757	*leu*	4	67	8.95	REGNERY (1947)
	10575	*tryp*	3	59	15.2	F. HUNGATE (1946)
	9185	*thi*	6	227	16.3	BELL (unpublished) & F. HUNGATE (1946)
	20705	*ad*	3	49	27.6	
	27663	"	6	152	11.2	
	70004	*ad-t*	9	173	18.2	
	605	*ser*				F. HUNGATE (1946)
D	263	*pyr*	5	227	5.72	
	35405	*pyro*	8	182	6.86	
	37803	"	10	262	10.3	
	39106	*pyro-p*	2	30	3.3	
	39706	"	2	27	7.4	
	44602	"	4	70	11.4	
	46904	"	2	30	1.6	
	44204	"	4	80	10.5	
	1298	*pyr*	2	27	14.8	
	37301	"	11	234	15.8	
	37815	*pyr-t*	8	255	11.2	
	44101	*pyr*	7	118	4.7	
	44302	"	1	18	5.5	
	45502	"	6	104	0.96	
	67602	"	6	171	10.5	
	67701	"	1	16	6.2	
	9666	*meth*	1	20	12.5	BUSS (1944)
	34486	*chol*	1	20	22.5	
	34542	"	4	119	25.2	
	37903	"	2	49	19.4	
	5531	*pan*	11	257	26.2	
	34556	"	1	15	20	
	38502	*pyr*	6	94	31.4	
E	33933	*lys*	3	52	4.8	GRANT (CALHOUN) (1945)
	16117	*isol-val*	12	235	8.9	
	47101	" "	1	20	5	
	35810	" "	1	18	8.3	
	39709	" "	1	12	12	
	46807	" "	1	14	10.7	
	36104	*meth*	1	20	30	BUSS (1944)
	37401	*inos*	9	196	22.7	
	83201	*inos-t*	2	142	31.7	
F	E15172	*sfo*				S. EMERSON unpublished
Unknown	C84	*hist*	1	10	15	
	517	*fat (1)*	1	23	2.2	
	1633	*pab*	4	81	29.6	
	1752	*fat (1)*	1	13	34.6	
	14789	*nitr*	1	15	33	

TABLE 6—(*continued*)

GROUP	MUTANT		NUMBER OF CROSSES	NUMBER OF ASCI	CENTRO-MERE DISTANCE	LINKAGE FIRST DEMONSTRATED BY
	28610	*ad*	4	81	14.2	
	28815	"	1	18	33	
	32213	*un-t*	1	18	36	
	33026	*val*	1	20	5	
	34547–10	*tryp*	1	33	18.1	
	35301	*ad*	2	33	15.2	
	39401	*nic-tryp*	6	118	23.3	
	40008	*tryp*	1	14	14.3	
	44008	"	1	18	11.1	
	44104	*thr*	2	35	18.5	
	44411	*ad*	5	85	34.1	
	44504	*pyr*	1	22	6.8	
	44601	*un*	1	21	0	
	45004	*ad*	1	20	20	
	45208	*un-t*	2	18	30	
	45304	*tryp*	2	43	9.3	
	45612	*un*	1	19	2.6	
	46407	*prol*	1	15	23	
	51505	"	1	16	28	
	46705	*ad*	1	16	25	
	47102	*pyr*	1	15	3.3	
	47305	*un-t*	1	18	27	
	47904	*chol*	5	71	31.3	
	66210	"	1	15	36.6	
	48504	*ad*	1	20	20	
	49001	*pyr*	1	17	5.8	
	50005	*thi*	1	20	27.5	
	63902	*pyr*	1	18	19.4	
	63904	"	2	43	14	
	68305	"	1	20	10	
	68902	"	1	22	20.4	
	71103	*un*	1	18	19.4	
	75001	*tryp*	1	19	13.1	
	83106	*un-t*	1	18	13.8	

Note: The data referred to, of BELL, BUSS, DOERMANN, GRANT, HUNGATE, REGNERY and SRB, are not included here.

17084 and 56501 show linkage to 18558 but in the case of 17084 much closer linkage is indicated. The two adenineless strains 27663 and 70004 behave as alleles, but 27663 shows much closer linkage to 44206 (the only crossovers observed were among random spore isolations) than does 70004.

Attempts to correlate LINDEGREN'S (1939) Group II with these linkage groups were not successful, possibly because the medium used was apparently not suitable for classification of most of the LINDEGREN mutants which were tried. *Fluffy* was used successfully but no linkage to the biochemical mutants

crossed to it was observed. Since it is not known whether any of the linkage groups which appear here correspond to LINDEGREN's chromosome II, letters rather than numbers have been used as designations. Group A corresponds to LINDEGREN's (1936) chromosome I. Group C was established by F. HUNGATE and REGNERY and is referred to by HUNGATE (1946) as chromosome III.

Genetic data which have appeared in earlier publications of the authors, and in the unpublished M.A. dissertation (Stanford University) of HOULAHAN, are included here.

SUMMARY

Data are presented from 330 crosses involving 118 mutants of *Neurospora crassa*. Spores were isolated, in order, from 8,549 asci, and in 6,905 of these germination was sufficiently complete to allow classification. Types of segregation observed are tabulated for each cross. Sixty-nine genes, representing at least 35 different loci, have been placed in linkage groups of which five are represented.

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MORPHOGENETIC STUDIES IN THE RABBIT. VI. GENETIC FACTORS INFLUENCING THE OSSIFICATION PATTERN OF THE LIMBS[1]

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IN recent years the study of the pattern of development of the skeleton, particularly of the time and order of appearance of the centers of ossification and of epiphyseal union, has been the object of intensive investigation, especially in man, because of the numerous practical applications of such knowledge in clinical procedures.

Many studies have been directed at the effects upon the skeleton of such environmental influences as disease, and vitamin and other nutritive deficiencies (FRANCIS 1939, 1940), but as early as 1907 PRYOR pointed out variations in the ossification pattern which are heritable as determined by the presence or absence of extra epiphyses. REYNOLDS (1943) found further evidence of hereditary differences in comparative studies of twins, siblings, cousins, and unrelated children. These studies have been very important in indicating the existence of genetic influences but they do not give a clear idea of the biological nature of such gene influences or how they operate and are transmitted. In order to determine their manner of action, it is necessary to have both the genetic constitution and the environmental factors as uniform as possible, which is a very difficult thing to do with human material.

The most promising material in which to study the relative importance of such influences and the precise manner and method of their transmission would be in races of animals rendered genetically uniform by close inbreeding and raised under as uniform a set of environmental conditions as possible.

The domestic rabbit appears to provide excellent material for this type of study as it manifests many variations in size, type, and conformation of body; and the development of inbred races affords an opportunity to isolate and evaluate the individual genetic components responsible for them.

In the present study, two inbred races of rabbits have been used for such analysis which are known to differ from each other with respect to the single gene causing dwarfism, to genetic differences in general body size, and to certain specific regional differences in body size.

The study shows that at least the first two have rather specific effects and

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because of the inter-relation of these factors, information is gained as to the process by which ossification of centers is initiated, progresses, and is influenced by them.

MATERIALS AND METHODS

Roentgenograms of the fore and hind limbs of 101 rabbits of two inbred races (III and X) were made during the first 20 days postpartum during which time most of the epiphyseal centers of the limbs are established. Race III is a sub-line of the New Zealand white averaging 3,800 grams adult body weight and inbred for 16–20 generations. Race X is a subline developed from CASTLE'S small A race averaging 2,200 grams. It is being maintained in the heterozygous condition for the dwarf gene (*dw*) which is sublethal when homozygous. The heterozygous animals are somewhat smaller than the normal ones and when

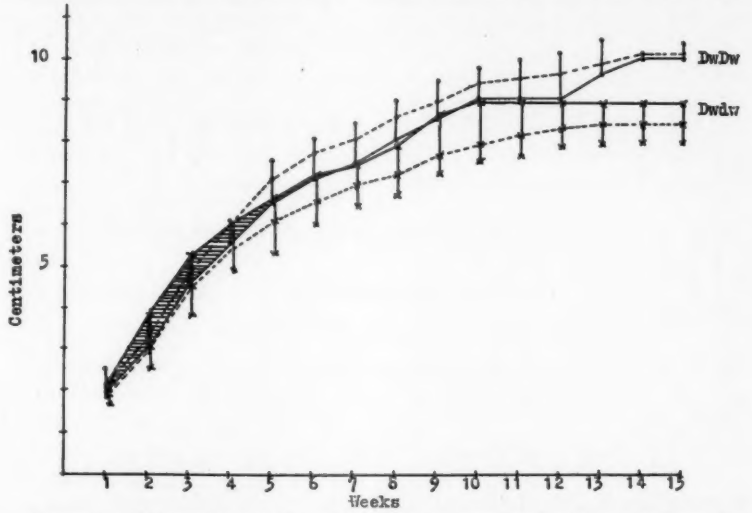

FIGURE 1.—Ear length of race *X* animals, means and extremes. Shaded area indicates overlapping of the two populations. Low extreme of *DwDw* and high extreme of *Dwdw* are indicated by solid lines, means by broken lines.

mated produce three kinds of progeny, normal, heterozygous, and homozygous dwarf, the last of which die within approximately three days. This segregation affords an opportunity to check on the differences between the effects of the dwarf gene (*dw*) and the racial growth genes. These differences are determined on the basis of ear length as it has been shown through breeding tests that animals heterozygous for dwarfism have significantly shorter ears. Heterozygotes are identifiable by their ear length after seven to eight weeks as shown in figure 1. Consequently, the animals have been raised to this age for absolute identification of this trait.

The animals were weighed and X-rayed at birth and on alternate odd numbered days for 19 days. X-rays were taken of the fore and hind limbs. With the

TABLE 1

Mean time and order of appearance of ossification centers.
Fore Limb

CENTERS	III				XDw				Xdw			
	NO.	$\bar{x}$	σ	C.V.	NO.	$\bar{x}$	σ	C.V.	NO.	$\bar{x}$	σ	C.V.
Distal Humerus	20	30.2	±0.62	2.05	14	31.4	±0.84	2.68	17	31.5	±0.94	2.98
Head of Humerus	20	31.8	1.32	4.15	19	32.6	1.06	3.25	30	33.2	1.44	4.34
Proximal Ulna	30	34.6	1.40	4.05	30	36.9	1.14	3.09	39	37.6	1.56	4.15
Distal Ulna	30	35.8	1.22	3.41	30	36.8	0.95	2.58	40	37.5	1.09	2.91
Distal Radius	30	35.8	1.22	3.41	30	36.9	1.01	2.74	39	37.9	1.26	3.32
Proximal Radius	30	35.7	1.23	3.45	30	38.1	1.28	3.36	39	38.9	1.47	3.78
Triquetral	30	37.3	1.31	3.51	31	39.4	1.20	3.05	39	40.0	1.16	2.90
Lunate	30	37.4	1.40	3.74	31	39.5	1.09	2.76	39	40.3	1.25	3.10
2nd Metacarpal	30	38.1	1.46	3.83	31	39.0	1.13	2.90	39	39.6	1.16	2.93
3rd Metacarpal	30	38.2	1.40	3.66	31	39.3	1.09	2.77	39	39.8	1.20	3.02
4th Metacarpal	30	38.4	1.41	3.67	31	39.7	0.94	2.37	39	40.4	1.39	3.44
5th Metacarpal	30	39.0	1.45	3.72	31	40.9	1.39	3.40	39	41.4	1.48	3.57
Navicular	30	39.6	1.28	3.23	31	41.6	1.28	3.08	39	42.4	1.29	3.04
Hamate	30	39.9	1.24	3.11	31	44.3	1.84	4.15	40	44.3	1.55	3.50
Greater Multangular	30	41.6	1.50	3.61	30	43.8	1.68	3.84	39	44.5	1.54	3.46
3rd Proximal Phalanx	30	42.4	1.30	3.07	31	43.5	1.31	3.01	40	44.1	1.57	3.56
4th Proximal Phalanx	30	42.5	1.36	3.20	31	43.4	1.31	3.02	40	44.2	1.68	3.80
2nd Proximal Phalanx	30	42.6	1.52	3.57	31	43.7	1.33	3.04	40	44.2	1.80	4.07
5th Proximal Phalanx	30	42.5	1.36	3.20	31	43.5	1.39	3.20	40	44.5	1.77	3.98
3rd Distal Phalanx	30	42.9	1.54	3.59	31	43.6	1.43	3.28	40	44.2	1.72	3.89
4th Distal Phalanx	30	43.0	1.61	3.74	31	43.7	1.41	3.23	40	44.3	1.64	3.70
2nd Distal Phalanx	30	43.2	1.65	3.82	31	43.7	1.55	3.55	40	44.4	1.68	3.78
5th Distal Phalanx	30	43.5	1.61	3.70	31	44.3	1.77	4.00	40	45.4	1.56	3.44

animal flat on his back and tied in position with thongs, the left fore limb was taped to the film holder, palm up with the ulna and humerus at a 45° angle. The hind limb was extended straight with the foot taped flat against the holder. Weights were taken when the animals were X-rayed and figures determined for the intervening days by interpolation.

It was found that when regrouped on the basis of copulatory age, the data gave more uniform results than it did when grouped according to age from birth because of the variation in the length of the gestation period (Av. 31 days, WING 1945).

Time of onset of ossification was determined by comparing the series of roentgenograms for each animal one with the other to ascertain the earliest possible appearance of the centers. First appearance was regarded as a faint diffuse shadow on the film. When a center appeared strongly developed one day, whereas two days previously there had been no trace of it, it was assumed that it first appeared on the intervening day.

In order to determine the rate of growth, measurements of the third metacarpals and metatarsals were made from the X-rays to the nearest $\frac{1}{4}$ mm with calipers and millimeter rule. By interpolation, the measurement for the intervening day was obtained. Widths of these same bones were also measured, with an ocular micrometer, converted to millimeters and treated as were the lengths.

Velocity curves were determined by plotting the daily increment against time.

OBSERVATIONS

1. Time and order of appearance of ossification centers

Time and order of appearance of the ossification centers of the limbs for each race are shown in tables 1 and 2. The variability of the centers is slight when compared with human studies (PYLE and SONTAG 1943) in part due perhaps to the shorter interval of time during which these centers appeared, and also to a considerable degree, we believe, to the genetic homogeneity of the population itself. They seem to fall into three distinct groups for each limb (separated by horizontal spaces, tables 1 and 2) with a time interval of from 2–5 days between them. If, however, the carpals and tarsals (in italics, tables 1 and 2) are disregarded for the moment, the remaining epiphyses then fall into four groups which are arranged according to the segments of the limb.

The order of first appearance in the three different populations is essentially the same except for short bones, but the time of ossification varies between the two races (III and X). The larger race appears to initiate ossification sooner in all centers. The irregularity of carpals and tarsals is in agreement with other investigations (PRYOR 1939; FRANCIS, WERLE and BEHM 1939; PYLE and SONTAG 1943).

An analysis of the first appearance of the centers in the three populations (table 3), as indicated by the t test (SNEDECOR 1946), shows the difference of the means between races III and X to be more highly significant than that between the two race X populations. The hind limb shows this most strikingly.

TABLE 2

Mean time and order of appearance of ossification centers.
Hind Limb

CENTERS	III				XDw				Xdw			
	NO.	$\bar{x}$	σ	C.V.	NO.	$\bar{x}$	σ	C.V.	NO.	$\bar{x}$	σ	C.V.
Distal Femur	20	31.0	±0.46	1.48	13	31.6	±0.87	2.75	17	31.5	±0.71	2.25
Proximal Tibia	20	31.8	1.20	3.77	15	32.7	0.97	2.97	24	32.7	1.11	3.39
Head of Femur	30	36.5	1.33	3.64	29	36.8	1.14	3.10	38	37.2	1.02	2.74
Distal Fibula	30	36.3	1.09	3.00	30	37.0	0.98	2.65	40	37.5	0.93	2.48
Distal Tibia	30	36.4	1.10	3.02	30	37.2	1.14	3.06	40	37.8	1.09	2.88
Navicular	30	36.6	1.07	2.92	31	38.6	1.23	3.19	40	38.6	1.53	3.96
Head of Trochanter	29	37.1	1.70	4.58	26	38.4	1.47	3.83	33	39.7	1.57	3.95
2nd Metatarsal	30	38.1	1.36	3.57	31	38.7	1.57	4.06	40	39.1	1.02	2.61
3rd Metatarsal	30	38.3	1.29	3.37	31	38.9	1.00	2.57	40	39.2	1.05	2.68
4th Metatarsal	30	38.6	1.25	3.24	31	39.4	1.05	2.66	40	39.8	1.40	3.52
3rd Cuneiform	30	39.0	1.19	3.05	31	43.1	1.70	3.94	40	43.5	1.88	4.32
Cuboid	30	39.3	1.31	3.33	31	43.3	1.69	3.90	40	43.7	1.94	4.44
5th Metatarsal	30	40.2	1.24	3.08	31	40.9	1.06	2.59	40	41.4	1.24	3.00
3rd Distal Phalanx	30	42.8	1.20	2.80	31	43.3	1.60	3.70	40	43.8	1.65	3.77
4th Distal Phalanx	30	42.8	1.35	3.15	31	43.4	1.58	3.64	40	43.8	1.74	3.97
2nd Distal Phalanx	30	43.0	1.31	3.05	31	44.0	1.74	3.95	40	44.3	1.62	3.66
2nd Proximal Phalanx	30	42.6	1.28	3.00	31	44.2	1.33	3.01	40	45.0	1.58	3.51
3rd Proximal Phalanx	30	42.7	1.18	2.76	31	44.1	1.34	3.04	40	44.9	1.64	3.65
4th Proximal Phalanx	30	42.8	1.21	2.83	31	44.0	1.33	3.02	40	45.2	1.48	3.27
5th Distal Phalanx	30	44.4	1.71	3.85	31	45.2	1.81	4.00	40	45.8	1.98	4.32
5th Proximal Phalanx	30	43.6	1.59	3.65	31	45.5	1.24	2.73	40	46.8	1.59	3.40
Proximal Fibula	30	45.4	1.45	3.19	31	46.9	1.27	2.71	40	48.0	1.50	3.12

TABLE 3

Significance of differences in time of first appearance of ossification centers between populations.

CENTERS	III AND X*Dw*	X*Dw* AND X*dw*
	t	t
Distal Humerus	4.64**	0.61
Head of Humerus	2.17*	1.39
Proximal Ulna	6.95**	2.01*
Distal Ulna	3.76**	2.68**
Distal Radius	4.02**	3.51**
Proximal Radius	7.75**	2.18**
Triquetral	6.49**	2.19**
Lunate	6.80**	2.49**
2nd Metacarpal	2.80**	2.16**
3rd Metacarpal	3.30**	1.94
4th Metacarpal	4.05**	2.33*
5th Metacarpal	5.06**	1.63
Navicular	6.26**	2.49*
Hamate	10.58**	0.26
Greater Multangular	5.43**	1.69
3rd Proximal Phalanx	3.24**	1.74
4th Proximal Phalanx	2.68**	2.36*
2nd Proximal Phalanx	3.03**	1.29
5th Proximal Phalanx	2.80**	2.64**
3rd Distal Phalanx	1.96	1.58
4th Distal Phalanx	2.00*	1.58
2nd Distal Phalanx	1.33	1.70
5th Distal Phalanx	1.67	2.25*
Distal Femur	2.67*	0.57
Proximal Tibia	2.30*	0.30
Head of Femur	0.98	1.66
Distal Fibula	2.63*	2.27*
Distal Tibia	2.88**	2.11*
Navicular	6.81**	0.00
Head of Trochanter	3.06**	3.17**
2nd Metatarsal	1.61	1.23
3rd Metatarsal	5.82**	1.15
4th Metatarsal	2.66**	1.40
3rd Cuneiform	10.97**	0.94
Cuboid	10.28**	0.93
5th Metatarsal	2.26*	1.98
3rd Distal Phalanx	1.53	1.10
4th Distal Phalanx	1.53	1.17
2nd Distal Phalanx	2.44*	0.82
2nd Proximal Phalanx	4.95**	2.18*
3rd Proximal Phalanx	3.73**	1.51
4th Proximal Phalanx	3.69**	3.37**
5th Distal Phalanx	1.68	1.51
5th Proximal Phalanx	5.18**	3.62**
Proximal Fibula	4.32**	3.36**

*=significant, $P<0.05$ **=highly significant, $P<0.01$ (see Snedecor, C. W. 1946)

Even in the third cuneiform and cuboid where the difference between the two races (III and X) is so marked, there is no significant difference between the race X populations. Since the variation in these populations is that associated with racial size (race III vs. race X) rather than the single gene (*dw*) difference in race X, it is particularly interesting to consider the growth differences of each population during this period.

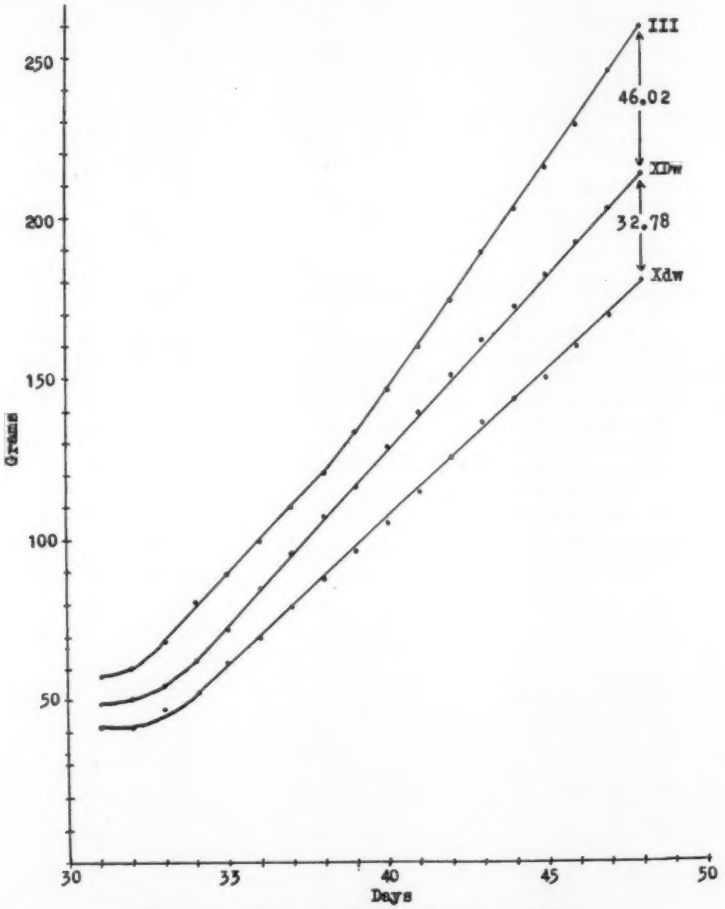

FIGURE 2.—Growth curves—days 30–50 post copulatory.

2. Growth differences

A. Body weight—The changes in body weight in the three populations of rabbits during the 20 day period are shown in figure 2. It is seen that at birth

TABLE 4

Significance of differences in weights, and lengths and widths of metacarpals and metatarsals.

DAY	III AND X*Dw* t	X*Dw* AND X*dw* t
	a. weights	
35	4.75**	4.21**
38	4.43**	5.50**
43	37.80**	5.17**
48	5.30**	3.11**
	b. Length of Metacarpals	
35	.87	3.02**
38	1.60	2.68**
43	.26	5.31**
48	.2	4.56**
	c. Length of Metatarsals‡	
35	.4	1.86
36	—	2.0*
38	.81	2.31*
43	.26	2.36*
45	—	2.06*
46	—	3.20**
48	.2	3.51**
	d. Width of Metacarpals	
35	2.0*	3.61**
38	5.57**	5.38**
43	4.76**	6.28**
48	6.56**	8.46**
	e. Width of Metatarsals	
35	0	7.07**
38	.87	7.38**
42	1.87	—
43	3.54**	7.40**
48	2.80**	9.90**

*=significant, $P<0.05$

**=highly significant, $P<0.01$ (see Snedecor, C. W. 1946)

‡ Since intervening differences are consistent (a, b, d), only four days are shown. Where changes do occur (c, e), additional days have been inserted.

there is already a marked difference between the three populations which persists and becomes increasingly divergent throughout the period. As shown by the t test (section a, table 4) these differences are statistically significant at every stage. Not only is race III larger than race X at birth, but the two groups of race X which differ from each other only in the presence or absence of the *dw* gene are also significantly different; and these differences become progressively greater.

While it is a well known fact that in adult rabbits the female is larger than the male, at the ages studied here the differences between the sexes are not significant. This is in agreement with CASTLE (1929) who found that the sex

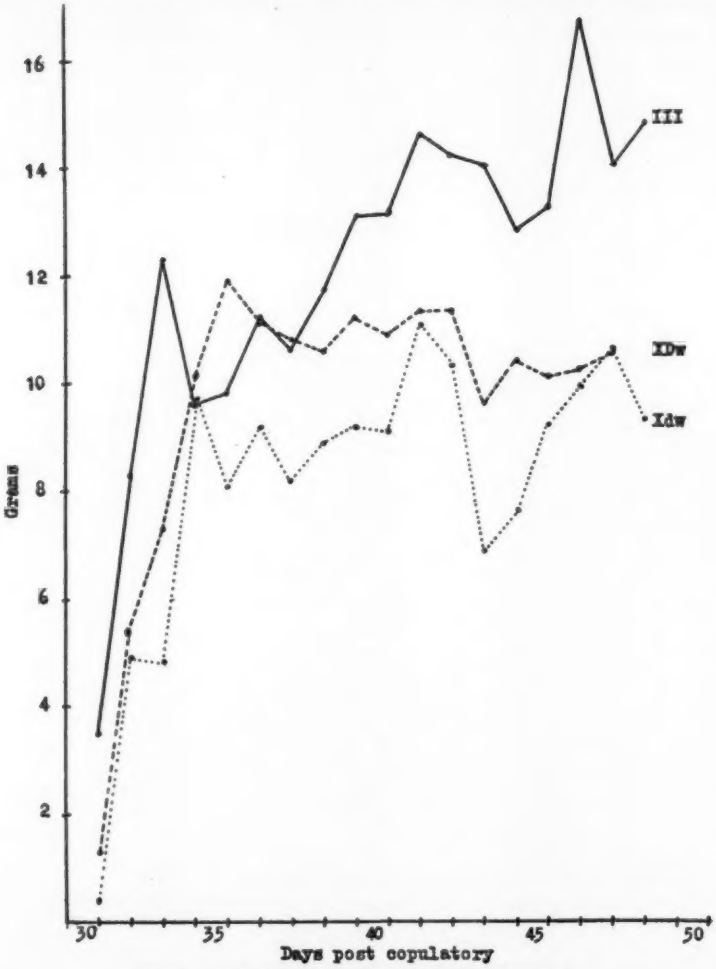

FIGURE 3.—Velocity of growth as determined by weight.

difference in body weight in the rabbit does not become manifest until the 40th day.

The velocity of growth as measured by weight is shown in figure 3. Race III animals grow very rapidly to the 34th day after which growth is slower, but the curve is still rising steeply, whereas race X *Dw* grows rapidly to the 35th–

36th day and race X *dw* only to the 34th–35th day. Thereafter both curves tend to level off. Thus, it is obvious that at approximately the 34th day there is a distinct change in the growth rate, which is markedly different in the two races (III and X). In race III growth is still proceeding but the velocity is retarded, whereas in race X the velocity becomes nearly constant.

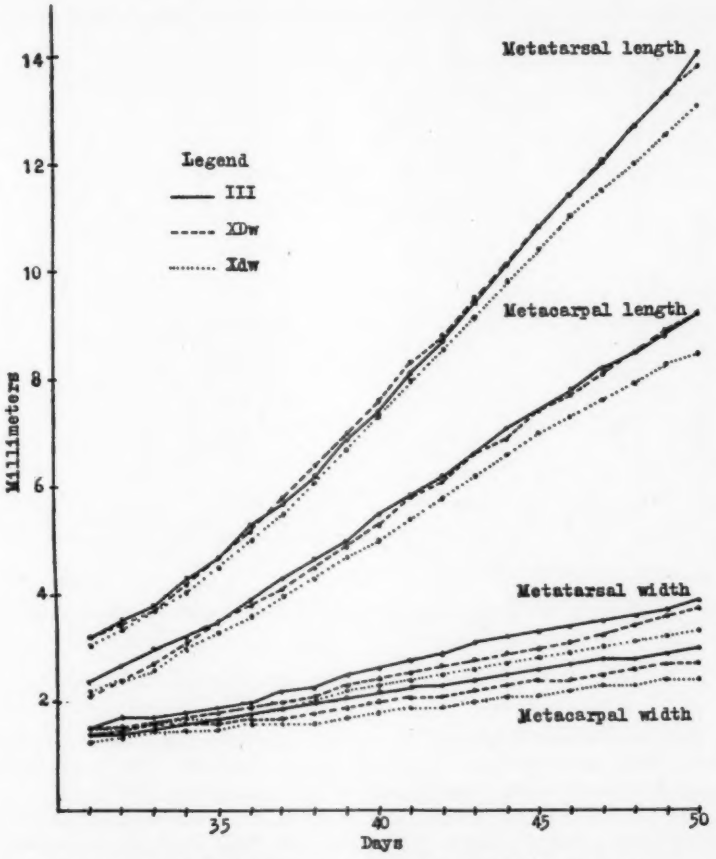

FIGURE 4.—Growth of metacarpals and metatarsals—days 30–50 post copulatory.

Since total body weight consists of growth increases in several organs and systems each of which can contribute differently to the total weight, it seemed desirable to look for the same growth changes in some structure more directly associated with and representative of the skeleton. Examination of the X-rays showed that of the available structures the metacarpals and metatarsals are undergoing a major portion of their growth during this period, and therefore,

are probably most reliable in this respect. The third metacarpals and metatarsals, being the largest, were selected for growth measurement. Changes in their length and width during the 20 day period are shown in figure 4.

B. Growth in length and width of the metacarpals and metatarsals—Casual examination of the roentgenograms gives the impression that the bones of race X animals are very slender as compared with race III. However, the statistical analysis of length and width of the metacarpals and metatarsals brings to light

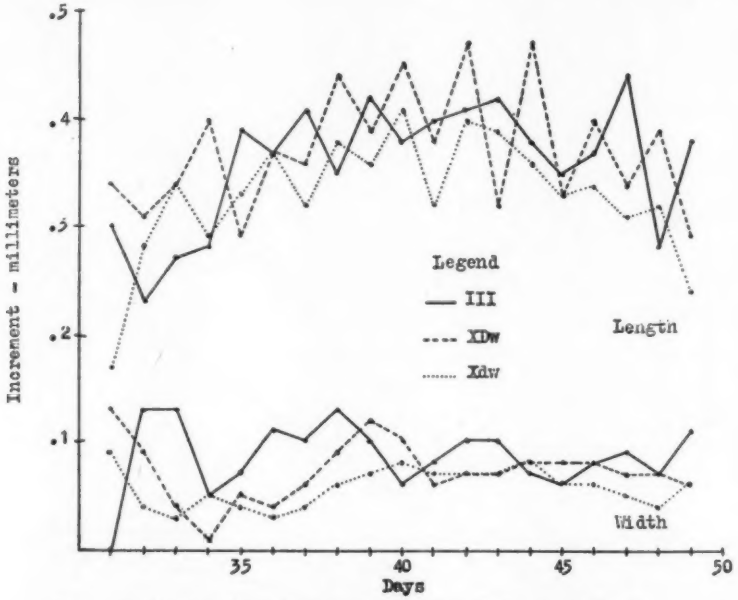

FIGURE 5.—Velocity of growth of metacarpals—days 30–50 post copulatory.

a most interesting relationship. In the first place, on the basis of length of these bones (sections b and c of table 4), the three populations are not as sharply separated as they are by body weight (section a, table 4).

The differences in metacarpals and metatarsals are not significant between the races III and X, whereas between the two race X populations the differences in metacarpals are significant throughout and the metatarsals from the 36th day on (table 4, c). Apparently the dwarf (*dw*) gene has a much greater influence upon longitudinal growth of the bones, once the centers of ossification have been established, than have the general growth differences between the two races.

The differences in width of metatarsals between races III and X (table 4, e) are highly significant at the later stages only (43–48 days) whereas those between the (*Dw*) and (*dw*) groups are highly significant throughout, indicating

that the effect of the *dw* gene upon transverse growth of bone is relatively greater than in length. Differences in widths of metacarpals (III and X *Dw*) are significant throughout, but again more highly so in the later stages. There

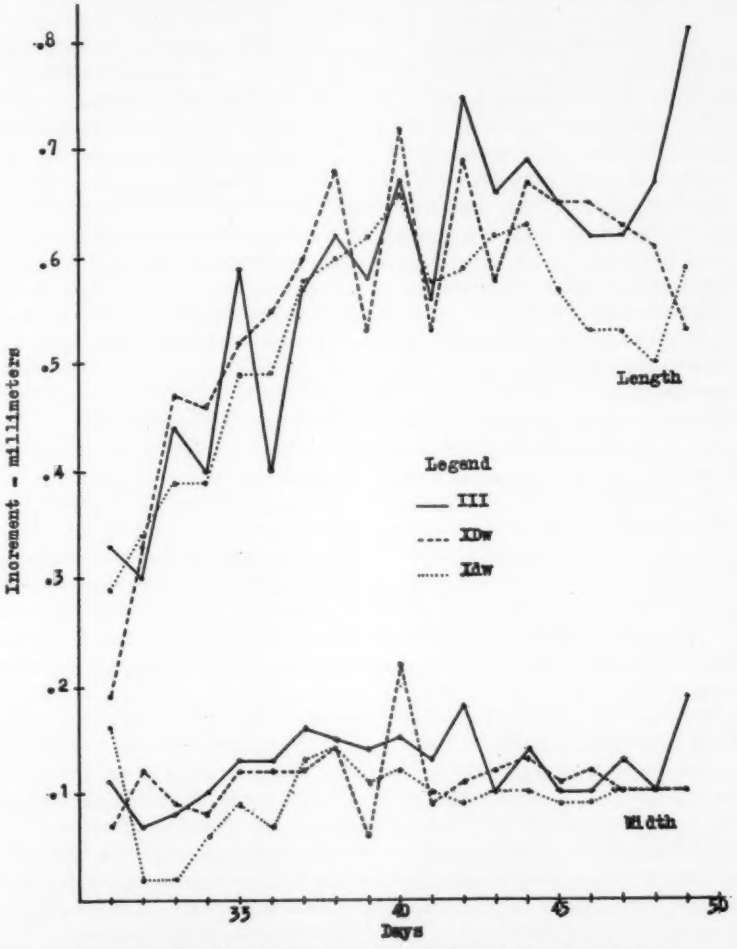

FIGURE 6.—Velocity of growth of metatarsals—days 30–50 post copulatory.

seems to be more indication of an effect of racial differences on width than there was on length. This effect is more manifest in the later growth stages. Velocity curves of these bones, both for length and width (figures 5 and 6), are concave downward, indicating a continuous decrease in velocity. The point of inflection varies slightly but is approximately at the 40th day in all cases.

Whereas velocity of growth as measured by weight decreased with a positive value, that of metacarpals and metatarsals changes from a positive to a negative value.

DISCUSSION

Reports of other investigators of time and order of appearance of ossification centers (PYLE and SONTAG, FRANCIS and WERLE, etc.) seem to agree that within any one species the order is essentially the same, but that variation does occur in time of appearance of the several centers. This is also true of the rabbit. Moreover, the present investigation particularly brings out the fact that genetic factors can influence the resulting ossification pattern in several ways. Both the general racial growth differences and the specific size gene (*dw*) produce effects which are recognizable by the methods of study employed here. They both seem to affect the time of appearance of the ossification centers generally since there is a tendency for each and all centers in the X*Dw* group to appear on the average one day later than in race III and for the X*dw* group to appear later than in the X*Dw* group. However, the effects of the racial differences are in general greater than those produced by the *dw* gene. The differences observed in the mean time of appearance of the entire ossification pattern appear to be greater in the fore limb than they are in the hind limb.

More specifically ossification begins later in the fore limb of the X*Dw* group than in race III and still later in the X*dw* group. In the hind limb it is earlier in race III than in X*Dw* but the difference is not as great and in the X*Dw* and X*dw* it begins at almost the same time. Thus the effects on the hind limb appear to be of the same nature but of a lesser magnitude, and that of the *dw* gene appears to be less than in the fore limb. It is interesting to note that this is the reverse of ossification of the ribs as described by BAUMGARTNER and SAWIN (1943). Ossification there begins earlier anteriorly in race X than in race III. Hence, ossification of the ribs and limbs is not controlled in quite the same way.

In contrast to the initiation of ossification the progress of ossification, once it has begun, as measured by the growth of the metacarpals and metatarsals, appears to be influenced more by the dwarf (*dw*) gene than by the general or racial genes. As indicated by the relative significance of the differences between the three groups, the racial differences appear to have little or no effect in the early stages but do begin to assume a greater influence as growth advances, and perhaps when more information is available they may be found to play a greater role in differentiation in later development at stages beyond the limits of the present investigation. The action of the dwarf gene on the other hand is expressed at all stages, including the earliest, and it appears to manifest a much more striking specificity of action. With respect to length of the metacarpal and metatarsal bones the retarding influence of the *dw* gene is greater in the fore limb than in the hind limb, corresponding to its effect upon first appearance of ossification. At the same time, width is less retarded by the *dw* gene in the fore limb than in the hind limb. This might be considered to be the result of a competition between the growth forces in the two directions except

for the fact that there appears to be no correlation in the magnitude of the differences observed and in the fact that the variations tend to be much greater in the later stages. In view of the tendency for these differences to be more significant in the later stages, and in view of the specificities described above, it seems reasonable to interpret these differences in progression of ossification as being due to variations in the time at which the racial or *dw* genes act and in the relative portion of the body which they affect. Consequently, we seem to have evidence that there are at least three distinct differences in the effects of the racial and dwarf *dw* genes.

While it has been pointed out that the order of appearance of the centers is the same in all three races, closer examination of tables 1 and 2 shows that in general the epiphyseal centers of the hind limb appear first in the region of the knee, then in hip and ankle, in foot, and then in the digits. In the fore limb, however, the centers of the humerus are first to appear, followed by those of radius and ulna, and so on progressing distally down the limb. This order is similar to all the preceding development of the limb, quite from limb bud formation through chondrification and ossification of the diaphyses (MINOT and TAYLOR 1905). Since otherwise the order of both limbs is the same in all three racial groups this difference between the fore and hind limbs seems specifically a species characteristic, and probably is the result of differentiation for the specialized habit of locomotion of the rabbit. The difference in grouping of the centers, however, serves to draw attention to the significance of such grouping. Such a phenomenon was first noted by FRANCIS, WERLE and BEHM (1939) who spoke of the tendency of the centers to appear in sheaves. However, the groups here are of definite and regular composition, whereas their sheaves were of "miscellaneous" constitution. It seems possible that if their time intervals of observation had been relatively as frequent as ours, that is perhaps weekly instead of every three months, their ossification centers might have fallen into a similar semblance of order as noted here. If this seemingly orderly grouping should correspond with the growth gradient of the embryologists (CHILD 1925; WEISS 1939), it is conceivable that the influence of such growth gradients might overlap, and therefore observations of appearance of ossification centers at too long intervals might result in the appearance of confusion in what might otherwise be an orderly arrangement.

In view of this point and the evidences previously obtained by SAWIN, *et al.* (see particularly SAWIN and NACE 1948) of regional centers of growth in the rabbit, it seems not altogether unlikely that the differences in length and width of metacarpals are also expressions of regional effects of the *dw* gene.

A further observation of some significance is the synchronization between the time of appearance of the secondary centers of the metacarpals and metatarsals (tables 1 and 2) with the point of inflection (PALMER 1924) of the velocity curves of ossification of the primary centers of these bones (figures 5 and 6). This observation is comparable to that of FRANCIS (1939) who stated that "children growing very rapidly tend to lag in epiphyseal rating unless they are provided with ample rations of vitamin D and minerals. When the period of rapid growth comes to a close, the epiphyseal rating advances

rapidly." It seems probable that if the velocities of ossification of other bones were to be determined, their epiphyses likewise would appear approximately at the point of inflection of the velocity curve. If this should prove to be the case, there would be provided a further clue to the processes of differentiation of the mammalian skeleton. This possibility is now under further investigation.

Although the present study appears to have raised more questions than it has answered, it clearly shows that genetic factors do have a large influence upon the ossification pattern, and that by the use of genetically uniform races, it is possible to differentiate in some degree specific gene influences. In this connection, it is further of interest to note where the specific genetic effects become manifest. The racial differences, which are directly comparable with the general size genes of CASTLE (1929, 1932 a, b; CASTLE and GREGORY 1929; GREGORY and CASTLE 1931), (in fact race X is directly descended from his original small race), appear to be manifested particularly at the extremes of the growth process, i.e., in the first appearance of ossification centers and towards the end of the ossification of the diaphyses, whereas the effects of the *dw* gene are more specific and occur during the time of most rapid growth. This is perhaps to be expected since general size was shown by CASTLE and GREGORY (1929) to be due to numerous factors acting in a more or less equal and cumulative manner and resulting in a generalized more rapid and sustained rate of cell division, whereas the dwarf gene has already been shown to have other rather specialized and specific influences (GREENE 1940).

SUMMARY AND CONCLUSIONS

The time and order of appearance of the secondary ossification centers of the fore and hind limb of 101 rabbits from two inbred races, III (30 animals) and X (71 animals), was obtained from roentgenograms taken on alternate days during the first 20 days postpartum. The two races differ from each other in adult body weight by approximately 1,400 grams. Forty of the race X rabbits, approximately half, were subsequently shown by the ear length and breeding test to be heterozygous for the dwarf (*dw*) gene. Neither the general (racial) growth genes nor the specific *dw* gene appear to influence the order of appearance of the centers since it is the same in all three populations. The time of appearance, however, is significantly different, the greatest variations being those associated with race. Progress of ossification, as measured by growth in length and width of the third metacarpals and metatarsals, also shows specific differences with the greatest manifestly those of the dwarf gene. Length is completely unaffected by the general size genes at this age. The centers appear in four distinct groups of definite constitutions which, in the fore limb, progress distally in regular order, whereas in the hind limb the order is modified by earlier differentiation of the knee centers, presumably associated with the specialized method of locomotion of the rabbit. Appearance of the epiphyses of the third metacarpal and metatarsal is accompanied by a definite slowing of growth. It is suggested that if this observation is substantiated by further study of other bones there will be supplied a valuable clue to the process of differentiation of the mammalian skeleton. No differences associable with sex were observed.

ACKNOWLEDGMENT

The authors are indebted to CAROLINE LYDECKER and JEAN WHITEHEAD for assistance in taking X-rays, and to DR. M. LEBOW whose interest and assistance stimulated the problem.

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NUMBERS AND DISTRIBUTION OF CHROMOSOME KNOBS IN UNITED STATES MAIZE

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THE chromosomes of maize possess a number of distinguishing features used by cytologists to identify members of the complement. Among the more important of these are the dark staining enlargements known as knobs, which have been shown to occur at certain points and only at certain points on the chromosomes. Although chromosome knobs are distinguishable in mitotic prophases they are best studied at the pachytene stage of meiosis at which time they are highly pycnotic when stained with ordinary chromatin dyes. Knob number is constant for any individual plant and in United States maize may vary from 0 in some varieties to 14 in others. It can therefore be used as one criterion in determining the relationships of various kinds of maize, a problem which is becoming increasingly important in modern maize breeding. Although this paper deals only with differences in total knob numbers there is some evidence to indicate that knob size and position might be equally useful as a tool in studying relationships in maize.

LONGLEY (1938) studied knob number and position in a collection of maize from 33 Indian tribes of the United States. He found very few knobs on the chromosomes of most strains from the northern Indian tribes. A slightly higher number of knobs were found in the southeastern varieties and many knobs were observed on the chromosomes of most varieties from New Mexico and Arizona. On the basis of these findings, LONGLEY suggests that "the number of knobs on the chromosomes of a strain of Indian corn may give a clue to the geographical origin of the strain."

MANGELSDORF and REEVES (1939) have suggested that the presence of chromosome knobs in maize is a result of admixture with Tripsacum, a related genus in which high knob number has been established for several species. They suggested further that the hybridization of Tripsacum and Zea with repeated natural backcrossing to Zea resulted in the formation of a new genus, Euchlaena. These investigators point out that so-called "Andean" or uncontaminated maize of the South American highlands has few or no knobs while most types of maize which show morphological evidence of Tripsacum introgression (either directly or through teosinte) have knob numbers roughly in proportion to the amount of putative Tripsacum germ plasm present. Further evidence supporting this hypothesis was offered by REEVES (1944) who, in a study of knob numbers in maize from North, Central and South America, demonstrated a statistically significant relationship between numbers of knobs and proximity to Central America, a region in which in certain areas both Tripsacum and teosinte occur in abundance. However, GRANER and ADDISON

(1944) found that *Tripsacum australe*, a South American species, unlike its Central and North American relatives, has no terminal knobs and few internal ones. These results suggest at least that knob numbers cannot always be taken as a reliable measure of the amount of Tripsacum germ plasm in Zea.

Disregarding the origin of chromosome knobs in Zea and their relation to the Tripsacum hypothesis they do nevertheless represent a stable, easily measured character. As will be shown later, differences in knob numbers and their association with morphological characters, seem to be significant, even in the highly selected maize of the United States corn belt.

MATERIALS AND METHODS

At the time this work was initiated the material used was limited to a series of inbred lines representative of corn belt dent corn. Later, it was felt that the true significance of the differences in knob numbers found in corn belt inbreds could not be fully appreciated without first having available some basic information on the parental types of maize that were combined to produce corn belt dents. For this reason, the field was expanded to include (1) Northeastern Flints, (2) Flints and Flours of the Northern Great Plains and the Midwest, and (3) Southern Dent Corn. A total of 171 strains have been studied representing a reasonably comprehensive collection of the major races of United States maize, exclusive of the southwestern United States and the coastal area of southeastern United States. The material has been studied over a period of three years, from 1945 through 1947. All varieties were grown in the field, the Southern Dents at Gray Summit, Missouri, and the Flints, Flours and Corn Belt Dents at Johnston, Iowa.

Knob numbers were determined from temporary smears of pollen mother cells at the pachytene stage of meiosis. For those inbreds included, most of which were relatively homozygous, sporocytes were collected from only one or two plants of each line. Among the open-pollinated varieties of Northern Flint and Southern Dent Corn, which, of course, were much more variable than the inbred lines, sporocytes were collected from two to four plants.

DESCRIPTION OF RACES

The 8 and 10-rowed flints of the northeastern United States, which were at one time the commonest type of corn in eastern North America, are essentially uniform morphologically (BROWN and ANDERSON 1947). These corns are characterized by long slender ears (fig. 1), wide crescent-shaped seeds, long and lax but usually few tassel branches, narrow leaves, many tillers and extensively developed husk leaves. The related varieties of the Northern Great Plains and the Midwest are in many instances very similar to those of the Northeast. In general, however, the flint varieties of the North Central United States are more variable than those of New York and New England. Among these corns one encounters higher numbers of rows of kernels, (fig. 1), more obvious mixture with flour and dent endosperm types, and a closer approach to Corn Belt Dents in plant type. In addition, many of these corns exhibit a series of characteristics which suggest a rather close relationship to certain Indian varieties of the Southwest.

The Southern Dents herein reported upon are quite variable and apparently have undergone a complicated history. Notwithstanding this fact, it is possible, on the basis of comparative morphology and known history, to separate them into two fairly distinct categories. These have been arbitrarily designated as (1) Old Southern Dents, and (2) Derived Southern Dents (BROWN and ANDERSON 1948). The former group is composed of varieties which are either very similar to some present-day Mexican corns or whose culture in the South dates back to Colonial times. Some of the varieties included in this category such as Mexican June and Tuxpan, for example, may actually be recent introductions from Mexico. With the exception of Hickory King, the ears of each of these varieties are typified by a very strong taper from base to tip. The strains which exhibit a high degree of denting tend to have high row numbers and considerable pointing. In practically all varieties of this category there is a strong tendency for the tip of the cob to extend well beyond the rows of kernels (fig. 2). The tassels although variable, usually have many secondary branches which are often highly condensed, particularly in the case of those varieties with high numbers of rows of kernels. Plants of Mexican June and similar varieties frequently have rather short, stiff, upright secondary tassel branches giving a pronounced "whisk broom" appearance to the tassel. The plants are tall, with as many as 24 nodes in some varieties. Ears are usually set high on the culms and internode length frequently becomes increasingly shorter above the ears.

The Derived Southern Dents differ from the former group primarily by exhibiting a more pronounced similarity to corn belt maize. The ears are usually more cylindrical; row numbers tend to be lower and there is a less pronounced tendency for exposed cob tips. Although plant types in the two groups show few consistent differences, it may be significant that the only variety encountered with highly pubescent leaf sheaths was among the Old Southern Dents. As a group the Derived Southern Dents are of somewhat shorter plant height than the Old Southern Dents. There are, however, among the derived sorts, certain varieties as tall as the tallest of the Old Southern Dents. Most varieties included in this category are apparently of more recent origin and are of more heterogeneous genetic background than the Old Southern Dents.

Since dent corn inbreds of the corn belt are widely grown and since they, as a group, are well known by most corn breeders and corn geneticists, it seems unnecessary to describe them in detail. The majority of the present day inbreds are the products of inbreeding open pollinated dent corn varieties of the cornbelt, or of inbreeding single or double crosses of corn belt inbreds. Since corn belt dent corn is itself a heterogeneous mixture resulting from the blending of Northern Flints and Southern Dents it is not surprising to find that corn belt inbreds range in type from strains resembling Northern Flints (fig. 3), to others approaching Southern Dent types, (fig. 4).

DISTRIBUTION OF KNOBS

It will be noted in tables 1, 2 and 3 that knob numbers in this material range from 0 to 12, and that numbers of knobs are closely associated with morpho-

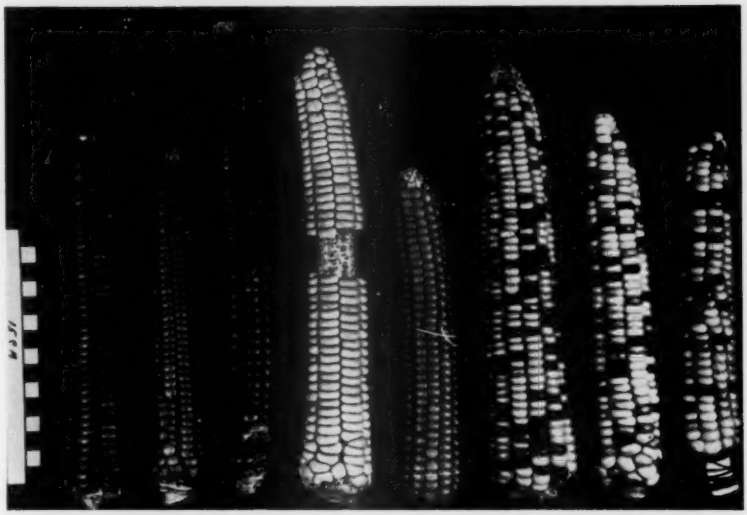

FIGURE 1.—Representative ears of flint and flour corns of the Northeast, Northern Great Plains, and the Midwest. Left to right. Stevens Flint—0 knobs; 8-row Flint (New York)—0 knobs; 8-row New England Flint—1 knob; New York Flour Corn—1 knob; Gehu Flint—0–2 knobs; 12 row Dakota Flint—3 knobs; Sax and Fox Flint—3 knobs; Bear Island Flint—2 knobs.

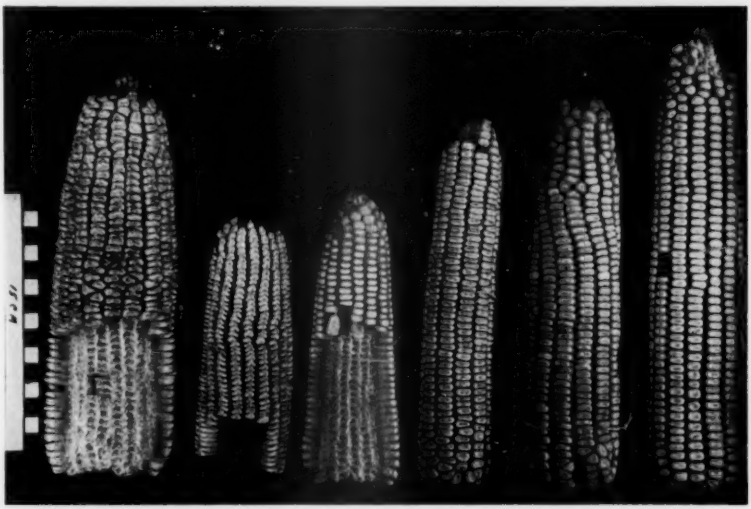

FIGURE 2.—Representative ears of "Old" and "Derived" southern dent corns. Left to right. Gourdseed—6, 7, 8 knobs; Shoepeg—6, 7, 8 knobs; Mexican June—7, 8, 9, 10, 11 knobs; Mosby's Prolific—5, 6, 7, 8 knobs; Clark's Yellow Dent—5, 6 knobs; Huffman—5 knobs.

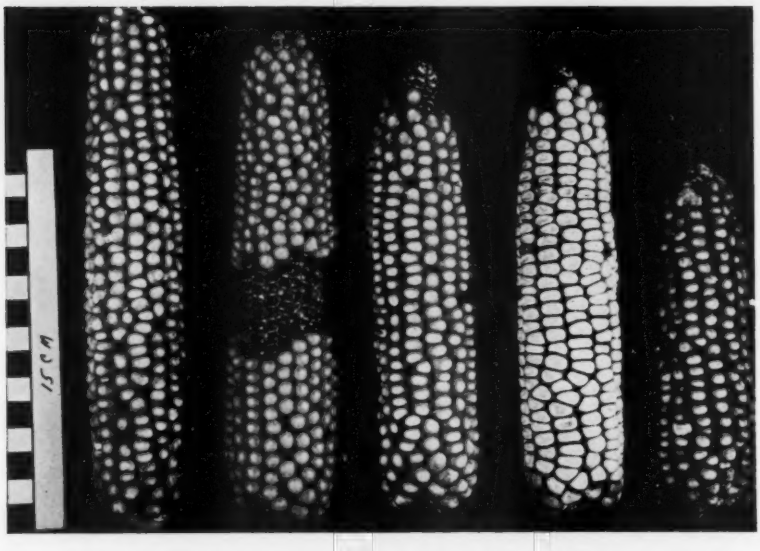

FIGURE 3.—Ears of inbred strains possessing low numbers of chromosome knobs. Left to right. L317—3 knobs; Ind. 38-11—3 knobs; I13—3 knobs; G1P1—1, 2 knobs; LDG—2 knobs.

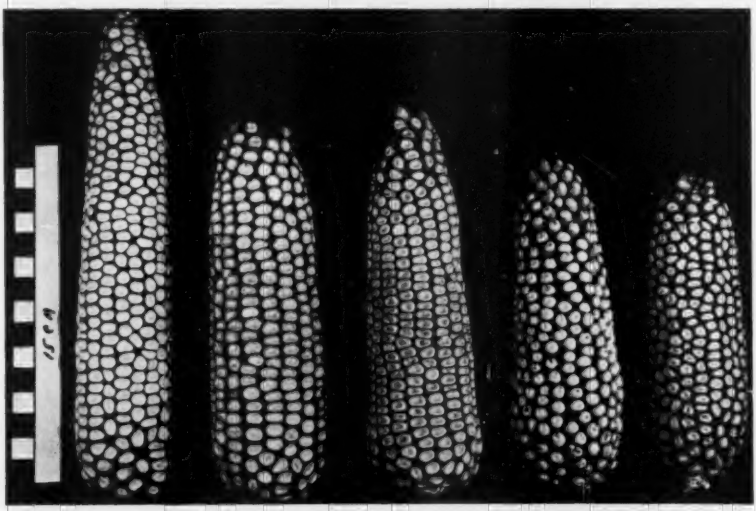

FIGURE 4.—Ears of inbred strains possessing high numbers of chromosome knobs. Left to right. Ill. R4—7 knobs; Os420—7 knobs; SI1—7 knobs; Ill. HY—6 knobs; L21—7 knobs.

logical type. The Northeastern Flints, for example, have few if any knobs. A number of these varieties have no distinct knobs and even the nucleolus organizer is frequently light-staining and indistinct. The maximum number of knobs in any of these varieties is 2, while several have but one knob, frequently

TABLE 1

Numbers of chromosome knobs in Northern flint and flour corn.

VARIETY	SOURCE	NUMBER OF CHROMOSOME KNOBS
Canada Flint	Feeding Hills, Mass.	0
Dutton	Newark Valley, N. Y.	2
Harris Mammoth Yellow	Rochester, N. Y.	0
Longfellow	Ontario, Canada	1
Mammoth Yellow	Ithaca, N. Y.	2
Parker's Flint	Potsdam, N. Y.	0
Quebec Flint	Restigouches, Que., Canada	1
Smut Nose	Bath, N. Y.	2
Stevens	Ithaca, N. Y.	0
Thayer Flint	Searsport, Me.	0
Thompson Flint	East Andover, N. H.	0
12-row Red Flint	Dryden, N. Y.	1
12-row Yellow Flint	Dryden, N. Y.	0
Wilbur's Flint	Hudson Falls, N. Y.	0
Assiniboine	North Dakota	1
Bear Island Flint	Minnesota	2
Dakota Squaw	North Dakota	1
Dakota White	North Dakota	0
14-row Dakota Flint	South Dakota	0
Gehu	North Dakota	0
Gehu	Iowa	2
Harris Mammoth Yellow	Iowa	0
Longfellow	Wisconsin	1
Mandan & Arikara	North Dakota	4
Mercer County Flint	North Dakota	0
Rainbow	North Dakota	1
Russian Extra Early	North Dakota	2
Russian Extra Early	Wisconsin	2
Sac & Fox	Iowa	3
Smut Nose	Wisconsin	1
Spanish Pop	Iowa	0
12-row Dakota Flint	South Dakota	3
Winnebago	North Dakota	1
Zuni Blue	North Dakota	5

at the terminal position on the short arm of chromosome 9. A wider range of knob numbers is encountered in the flint and flour varieties of the Northern Great Plains and the Midwest. Among these corns, numbers range from 0 to 5. Variation in numbers of knobs is here accompanied by considerable variation in morphological type.

The highest number of knobs encountered in this study were among the Old Southern Dents. Numbers in these varieties range from 5 to 12, with a rather strong mode at 7 to 8. Eleven and twelve knobs were found only in June Corn, which is of Mexican June type, and the variety Hickory King. Each of these corns are similar to some present-day Mexican varieties which are known to

TABLE 2

Numbers of chromosome knobs in Southern dent corns.

VARIETY	SOURCE	NUMBER OF CHROMOSOME KNOBS	VARIETY	SOURCE	NUMBER OF CHROMOSOME KNOBS
Gourdseed	Texas	6, 7, 8	Southern Snowflake	Virginia	5
Hickory King	Virginia	7	Southern Yel. Dent	Mississippi	4, 5
Hickory King	Georgia	6, 11, 12	Strawberry	Texas	8, 9
Hickory King	Tennessee	5, 7, 8	Va. Horsetooth	Virginia	4, 6, 7
Jellicorse	Virginia	9	Whatley's Prolific	Virginia	7, 8
June Corn	Louisiana	10, 11	Yellow Dent	Louisiana	6
Mexican June	Tennessee	7, 8	10-Row Dent	Arkansas	5
Mexican June	Tennessee	9	Cambren	Kentucky	6
Old White Dent (1)	Arkansas	5	Caraway's Prolific	Louisiana	6, 7
Old White Dent (2)	Arkansas	5	Cherokee	Georgia	5
Red Cob Chisholm	Texas	10	Clarks Yel. Dent	Texas	5, 6
Shoepeg	Louisiana	7, 8	Columbia Beauty	Tennessee	6
Shoepeg	Louisiana	6	Garretts 1	Kentucky	5, 6
Shoepeg	Louisiana	7	Garretts 2	Kentucky	7
Tenn. Red Cob	Tennessee	7, 8	Giant Yel. Dent	Texas	5
Tuxpan	Louisiana	7, 8, 9	Huffman	Tennessee	5
Tuxpan	Virginia	8	Jarvis Golden Prolific	Tennessee	5
White Dent	Arkansas	7	Jarvis Golden Prolific	Mississippi	4
Yellow Shoepeg	Louisiana	7, 8, 9, 10	Johnson Co. White	Missouri	7
Yellow Tuxpan	Louisiana	8	Latham's Double	Virginia	6
Neal's Paymaster	Arkansas	6	Mammouth Ensilage	Virginia	5
Neal's Paymaster	Mississippi	6	Mosby's Prolific	Mississippi	5
Sherman	Tennessee	5	Mosby's Prolific	Virginia	7, 8
Southern Ill. 1	Illinois	5	Mosby's Prolific	Louisiana	7
Southern Ill. 2	Illinois	6, 7	Mosby's Prolific	Tennessee	6

possess rather high numbers of chromosome knobs. It seems quite probable therefore that the Mexican June and Hickory King types of corn of the southern United States have come directly from Mexico without having undergone much change in plant or chromosome morphology. It is likewise relatively easy to find in central Mexico today varieties which closely approach in ear type the southern Gourdseeds and Shoepegs.

Knob numbers in corn belt inbreds range from 2 to 8 and their overall distribution is almost exactly intermediate between that of the Southern Dents and Northern Flints (fig. 5). This distribution, it would seem, might well be

TABLE 3

Numbers of chromosome knobs in dent corn inbreds.

NO. CHROMOSOME KNOBS	INBRED STRAIN
1	GIPI*
2	B4AM1*, CI.61, G1P1*, Ind. 33-16, IW1*, JC3*, KG2*, K77AT1*, LDG, LE4*, SW19*, S32*, W61*, 101*.
3	A94*, BG6*, B4Y3*, CI.7, CI.61, Conn. 103, D31*, F3-2, G3K2*, I13*, JG3*, J61*, KYS, K43*, L24*, L317, Minn. 49, Ohio 28, Ohio 40b, SN2*, SW19*, W23, 38-11.
4	AE1*, AG1*, B4U2*, CI.187-2, Conn. 103, Conn. 105, IDT, Ind. B2, Ind. Lx2, Ky27, K4, K77, LE7UI*, Minn. 11, SO2*, SRS386*, T27GSA*, 104*, 106*.
5	A99*, CI.7, CI.187-2, F3K4*, Ill. M14, Ind. B2, IW1CM2*, K155, K201, LF5*, MD1*, MY1*, SO1*, SS2*, SY1*, WF9, WG1*, WP1*, 103*, 105*.
6	BH1*, BH2*, CI.2, CV1*, Ill. A, Ill. HY, LA, SS2*, W26, 102*.
7	F3B2*, H65*, Ill. R4, L21*, Os 420, SI1*, SK2*.
8	GN2BD1*.

* Inbred Strains developed by PIONEER HI-BRED CORN COMPANY.

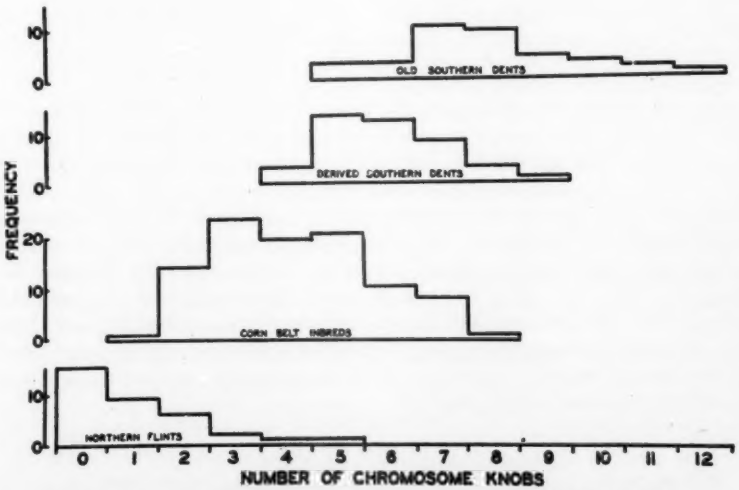
FREQUENCY
OLD SOUTHERN DENTS
DERIVED SOUTHERN DENTS
CORN BELT INBREDS
NORTHERN FLINTS
0 1 2 3 4 5 6 7 8 9 10 11 12
NUMBER OF CHROMOSOME KNOBS

FIGURE 5.—Frequency distribution of chromosome knobs in United States maize.

taken as further substantiating evidence for the hybrid origin of corn belt maize in which the two parental types are represented by the Northern Flints and Southern Dents.

RELATIONSHIP BETWEEN NUMBERS OF KNOBS AND MORPHOLOGY

Even a cursory survey of figures 1 to 4 will suggest that numbers of chromosome knobs in United States maize do not vary at random but are instead associated with several morphological characters. A detailed statistical analysis of these relationships is being made and will be published at a later date. For this reason only the following more obvious associations between knobs and morphological characters will be referred to in this report.

Number of Rows of Kernels

Since the 8-rowed Northern Flints have but few knobs and the many-rowed Southern Dents have several, one might expect to find some correlation between knobs and row number in the maize of the corn belt. A positive correlation between these characters does exist although the value is not high. For 95 dent corn inbreds the correlation between knobs and row number is $r = +.20$ (significant at .05 level). For all the material herein reported upon including Northern flints, Southern dents and corn belt inbreds the correlation between these characters is $r = +.73$.

Denting

For purposes of this study denting has been scored in the following manner:

0. No soft starch at crown of kernel
1. Soft starch but no denting
2. Soft starch and a small dent
3. Soft starch and a deep dent but no wrinkling of pericarp
4. Soft starch and wrinkling pericarp
5. Soft starch and the apex of the kernel collapsed

In a population comprising the Northern Flints and Southern Dents there is, of course, a very close relationship between knobs and denting. As is true in the case of row number some association between these characteristics still exist in corn belt inbreds. For 95 dent inbreds the correlation coefficient between knobs and denting is $r = +.20$, which value is significant at the 0.5 level. Even though this correlation is low it is perhaps significant that there should be any relationship between these characteristics since corn belt corn has undergone at least a century of very intensive selection for soft texture. The genetics of denting is complicated and is controlled by a number of genes which are probably distributed over several chromosomes. It is interesting, therefore, to find such a complex quantitative character associated with knobs, a factor which certainly has not been subject to intentional selection.

Husk Leaf Blades

Data on number and length of husk leaf blades ("flag leaves") are not available for all cultures included herein. Nevertheless, observations on a number

of varieties of Northern Flint and Southern Dent corn and approximately 75 dent inbreds reveal that flag leaves are closely associated with few or no knobs. Flag leaves are rarely found in Southern Dents but are extensively developed in most varieties of Northern Flints. This relationship carries over into corn belt inbreds. Those strains with longest flag leaves are found among the lowest knob groups whereas few strains with well-developed flag leaves have been found among inbreds with high numbers of knobs. It seems likely therefore that flag leaves are very closely associated with absence of knobs since more than a century of breeding and selection has failed to alter this association significantly.

Seminal Roots

While it is recognized that seminal root development in corn is greatly influenced by age of the seed and environmental factors, it is also apparent that there are inherent differences in numbers of seminal roots produced by different varieties of United States maize. In the Southern Dents the mean number of seminal roots per seed was found to be 4.70. The Northern Flints averaged 2.40. The average number of seminal roots for inbred strains in the 1 to 4 knob group was 2.54 whereas inbreds in the 5 to 8 knob group averaged 3.64. Thus there appears to be a rather close association between knobs and seminal roots in the open-pollinated Northern Flints and Southern Dents and some slight association between these characters in corn belt inbreds.

Ear Shape

The overall shape of the ear of maize is difficult to measure accurately. The ear may be cylindrical, it may be tapered from base to tip or it may be tapered from center to tip and at the same time contracted at the base. During this study it became evident that most strains could be placed in one of two categories (*pyramidal* or *cylindrical*) so far as shape of the ear is concerned. Although it is recognized that this classification is somewhat crude and not absolutely reliable, it has been found to be usable. Early in this study it became apparent that the knob number of corn belt inbreds could be predicted with some accuracy by observing the shape of the ears. It was found, for example, that if a strain possessed pyramidal ears the chances were good that it would have five or more knobs. If on the other hand the ears of an inbred were cylindrical or nearly so, it was usually found to have fewer than five knobs. The reasons for this association in corn belt material were clear when the Northern Flint and Southern Dent corns were analyzed. The ears of most Southern Dents are distinctly pyramidal whereas the low knob Northern Flints are typified by cylindrical ears. This association strongly expressed in the ancestors of corn belt corn has been maintained even in the highly selected inbred parents of today's hybrid corn.

Irregular Rows

Irregular rows of kernels covering usually a portion of the ear only are frequently found in the Southern Dents and certain high knob inbreds. Since these corns are basically straight rowed the occurrence of irregularity is the

result of some breakdown in the usual arrangement. An examination of a large number of ears suggests that at least three types of irregularity occur. That which is observed rather frequently is an irregular arrangement of rows due to the abortion of one member of a pair of paired spikelets. Another common occurrence is the dropping of a row or several rows of paired spikelets. This type of phenomenon seems to occur more frequently in the Southern Dents than in corn belt inbreds. A third type of irregular rowing and one which has been observed less frequently is that resulting from multiplication (CUTLER 1946).

The Northern Flints and dent inbreds with low numbers of rows of kernels tend to have very straight rows. There are, however, a few exceptional cases among these varieties in which a tendency toward irregularity does exist. In the majority of such cases examined the irregular rowing was found to be due to multiplication.

There is, therefore, in this material a tendency for irregular rows to occur in combination with high numbers of chromosome knobs. However, this association would be altered if the Caribbean flints of the Gulf Coast and the Indian varieties of southwestern United States were included in the survey. These two types of corns are in general typified by high numbers of knobs and straight rows, an association that is exactly the opposite of that found in the Southern Dents, Northern Flints and corn belt inbreds.

DISCUSSION

Data on knob numbers in maize are now available for a rather large number of varieties of South, Central and North America. Only for a collection of Guatemalan corn, however, has information on knob numbers been correlated with morphological data. MANGELSDORF and CAMERON (1942) have shown that for 162 varieties of Guatemalan maize there is an association of high and low numbers with several plant and ear characters. Their data demonstrate quite definite associations between high knob number and cylindrical *vs.* pyramidal ears, straight rows *vs.* irregular rowing, denting *vs.* flint and flour endosperm, glabrous leaf sheaths *vs.* pubescent sheaths, and several other plant and ear characteristics. Although our present knowledge of corn in the western hemisphere may not yet permit a fair comparison of United States maize with that of Guatemala, it is interesting to note that only in two instances do the results of this study conflict with the correlations reported by MANGELSDORF and CAMERON. High numbers of knobs in the United States material included in this study are positively correlated with irregular rowing and not with straight rows. Among United States corns those varieties with most consistent straight rows are found in the Northeastern Flints with very few knobs while the strongest tendency for irregular rowing occurs in certain Old Southern Dents and high knob inbreds of the corn belt. The second case in which these data conflict with the observations on Guatemalan corn is that of pubescence. Although pubescent leaf sheaths are not commonly found in United States maize, it is interesting to note that the only variety included

here which exhibited marked sheath pubescence was Tuxpan, one of the Old Southern Dents with 7, 8, and 9 knobs. The most glabrous varieties of United States corn are found among the almost knobless Northeastern Flints.

It is not probable, however, that this bit of conflicting evidence has any great significance. Our present knowledge of United States corn suggests that Guatemalan varieties have played a very minor role in the origin of United States varieties. Although we cannot yet completely abandon the idea of a Guatemalan origin of Northern Flints the evidence for such a hypothesis is indeed meager. In contrast, there is abundant evidence that a large segment of United States corn (most southern varieties exclusive of Caribbean flints) has stemmed directly from Mexico, and since Mexican and Guatemalan maize are very different entities it is probably incorrect to make direct morphological and cytological comparisons between Guatemalan and United States varieties. The work of MANGELSDORF and his collaborators suggests that the presence of chromosome knobs in maize is the result of contamination with Tripsacum. If this hypothesis can be applied to United States corn one would expect the Northern Flints, which externally exhibit more Tripsacum influence than any other group of varieties included in this survey, to possess many knobs. Actually, they have fewer knobs than any other group of United States maize. This may mean one of two things. Either the assumed tripsacoid nature of the Northern Flints is a superficial one or these flints are the result of the admixture of a knobless maize with a low knob Tripsacum, such as *T. australe*. It would seem, therefore, that more data are needed before one can safely regard knobs as a reliable indicator of Tripsacum germ plasm in Zea. It does appear, however, that knobs may represent one of the better indicators of relationships in corn.

SUMMARY

1. Chromosome knob numbers were determined for representative collections of United States maize consisting of Northern Flints, Southern Dents, and corn belt inbreds.

2. Knob numbers range from 0 to 12 with the greatest number being concentrated in the Southern Dents, the lowest in the Northern Flints. The distribution of knobs in inbreds of the corn belt is intermediate between the Northern Flints and Southern Dents.

3. High numbers of knobs are positively correlated with the following external features of the ear and plant: High row numbers, denting, absence of husk leaves, many seminal roots, and irregular rows of kernels.

4. The fact that similar relationships between knobs and morphological characters can be shown in the open pollinated Northern Flints and Southern Dents and in corn belt inbreds suggests that associations present in the ancestors of corn belt maize have not been extensively altered even after a century of breeding.

5. The present data are discussed briefly in light of the Tripsacum hypothesis. It is suggested that more data are needed before one can safely use knob numbers as an index to the amount of Tripsacum germ plasm in Zea.

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A CYTOLOGICAL SURVEY OF WILD POPULATIONS OF TRIMEROTROPIS AND CIRCOTETTIX. (ORTHOPTERA, ACRIDIDAE). I. THE CHROMOSOMES OF TWELVE SPECIES

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INTRODUCTION

IN RECENT years a great deal of work has been carried out on the distribution of structurally different chromosomal types in wild populations of Drosophila (DOBZHANSKY 1947 a, b, c, 1948; DOBZHANSKY and EPLING 1944; WARTERS 1944; CARSON and STALKER 1947). Similar studies have also been carried out on some species of Chironomus (PHILIP 1942; HSU and LIU 1948). Comparisons have been made between different populations of the same species and between samples of the same population collected at different seasons of the year and in several successive years.

These studies have been almost entirely concerned with one particular type of chromosomal rearrangements, namely paracentric inversions. In the case of *Drosophila pseudoobscura*, the species which has been studied most extensively, it was found by DOBZHANSKY that individuals heterozygous for inversions were, as a rule, better adapted under natural conditions than either of the two homozygous types. Thus, if we call one gene-sequence A and the other A', individuals of the constitution AA' possessed a higher degree of fitness than either AA or $A'A'$ individuals, although the relative fitness of the various types showed regular seasonal fluctuations. Results such as these (which can be duplicated in laboratory experiments carried out under controlled environmental conditions in "population cages") explain the existence of the multiplicity of different gene-sequences known for the chromosomes of *D. pseudoobscura* and some other species of the genus Drosophila. Thus, in the example we have chosen, natural selection will never lead to the extinction of either sequence A or A', as it might if the viability of the heterozygotes were intermediate between the viabilities of the two homozygous types or lower than either of them.

Heterozygosity in respect of paracentric inversions is not, however, the only type of cytological heterogeneity present in natural populations of animals. In the grasshoppers such inversions seem to be completely absent in the wild populations of most species, doubtless because they would cause some degree of sterility in a group where chiasma formation occurs in the male.[1] Neverthe-

[1] The only authors who have found inversions in grasshoppers are DARLINGTON (1936), who found typical "inversion bridges" and acentric chromosome fragments in several individuals of *Chorthippus parallelus* and *Stauroderus bicolor*, and COLEMAN (1947) who states that they are common in grasshoppers, without giving any details.

less, in one group of grasshoppers a type of structural rearrangement is found in the populations of many species which is completely unknown in Drosophila. The group in question consists of the Trimerotropis-Circotettix-Aerochoreutes complex of genera which belong to the sub-family Oedipodinae (Banded-wing grasshoppers) and the structural rearrangements are transpositions ("shifts") of the centromere. Such populations were studied by the earlier workers on orthopteran cytology (CAROTHERS, KING, HELWIG) during the years 1914–1929, but these authors were concerned, in the main, with using this extremely favorable material to test and prove the independent segregation of the homologous chromosomes at meiosis. Only in the work of HELWIG (1929) do we find an approach towards a study of the population-dynamics of these cytologically polymorphic species of grasshoppers.

In the light of the extensive studies of DOBZHANSKY and the theoretical work of WRIGHT it now appears that it would be worth while to re-investigate the cytology of the natural populations of these Trimerotropine grasshoppers, with a view to determining the frequencies of the various chromosomal types, whether significant differences in respect to these frequencies exist between geographically remote or isolated populations, and whether these frequencies are determined by the same general principles as seem to govern the distribution of inversions in Drosophila populations. Such a task will necessarily involve several years' work and the present paper is merely a preliminary discussion of the problem, reporting briefly on the cytological conditions encountered in the eleven species of the group which we succeeded in collecting during a month's trip (June 1948) through New Mexico, Arizona, Nevada, Utah and Colorado, and one species which we studied several years ago.

TAXONOMY OF THE TRIMEROTROPINE GRASSHOPPERS

Of the three genera to be considered in this series of papers, Trimerotropis is by far the largest and, for reasons to be discussed below, was probably ancestral to Circotettix and possibly also to Aerochoreutes. With the exception of two species found in South America (one of which is endemic in Chile) it is confined to North America, ranging from Canada to the state of Oaxaca at the extreme southern end of the Mexican central plateau. It has been recently estimated (BALL, TINCKHAM, FLOCK and VORHIES 1942) that 43 species of Trimerotropis exist in N. America, but since the taxonomy of some sections of the genus is still in a confused state, this estimate is necessarily a rough one. Many of the species show extensive geographical variation so that in order to be certain of the existence of discontinuities between one form and another it is necessary to examine individuals from a large number of localities covering the whole range of the forms in question. To describe a new species, even on the basis of a large series of individuals *if these are all from a single locality*, is clearly unsafe in the case of many sections of Trimerotropis, since one may be dealing merely with a local variant of some previously recognized species. Many of the "species" of MCNEILL (1901) are synonyms created as a result of errors of this kind. In this respect the Trimero-

tropine grasshoppers are a complete contrast to Drosophila, where the external morphology of the species shows little geographical variation.

The majority of the forty-odd species of Trimerotropis are confined to the western half of the U. S. A. Only four occur east of the Mississippi; these are *T. maritima* Harris, which occurs on sandy ocean beaches from Maine to Florida, and which is represented by a distinct subspecies, *T. maritima interior*, along the shores of the Great Lakes; *T. acta* Hebard, from Southern Florida, *T. citrina* Scudder which ranges from Maryland to the Pecos river in Texas and New Mexico; and *T. saxatilis* McNeill which is found from N. Carolina to Arkansas and northeast Texas. A fifth eastern species is *T. schaefferi* Caudell, from the Gulf Coast of Texas. The remainder of the species are mainly confined to the Rocky Mountains, Intermontane Plateaus and Pacific Mountain System, although *pistrinaria* and *pallidipennis* extend into East Texas. The extent to which members of the genus occur in northern Mexico is only imperfectly known (*pallidipennis* ranges over most of the Mexican central plateau, and several species have been recorded from Baja California). Most of the Trimerotropi are essentially grasshoppers of arid regions and in the eastern U. S. A. they are only found on rocky hillsides (*T. saxatilis*), sandy coastal areas (*T. maritima*, *T. acta*, *T. schaefferi*) and other arid localities (*T. citrina*).

On cytological grounds we may divide the genus Trimerotropis into two sections. In the first of these are those species in which all the chromosomes (including the X) are acrocentric (rod-shaped). Since this is the condition which is almost universal in the Acrididae (except for a few aberrant genera) it is clear that these are the primitive species of the genus, in which the centromeres of all the chromosomes still retain an almost terminal position. We have previously (WHITE 1945) mentioned *T. maritima* and *T. citrina* as belonging to this group; to these COLEMAN (1948) has added *T. fontana* and *T. praeclara* and we are now able to add three more species (see below). We shall refer to this group of species as section A of the genus; it seems undesirable to create a named subgenus until it has been shown that all the species with acrocentric chromosomes form a taxonomically compact and homogeneous group.

Section B comprises those species of Trimerotropis in which some of the chromosomes have become metacentric (V-shaped). Since no reduction in chromosome number has taken place (except in *T. cyaneipennis*, which has 21 chromosomes in the male instead of 23) it is clear that these metacentric elements have arisen from acrocentric ones by some sort of shift of the centromere (that is, a homosomal rearrangement in the terminology of MULLER (1940)). The exact number of metacentric elements varies within group B from species to species, and in some species from individual to individual. In all species hitherto studied, however, the X chromosome is a metacentric; it must therefore have undergone a centromere shift early in the phylogenetic history of Section B.

Where the number of metacentric chromosomes varies from individual to individual it is clear that we have a rather complex situation in the natural populations of the species. A particular chromosome may be represented by

two acrocentric elements in one individual, by an acrocentric and a metacentric in another and by two metacentrics in a third. In most of the species of group B there are some chromosomes which are invariably metacentric and some which are sometimes metacentric and sometimes acrocentric; but group B also includes some species which show no cytological variation from individual to individual, certain chromosomes being invariably acrocentric, others invariably metacentric. We may call the latter cytologically monomorphic species, the former cytologically polymorphic.

The genus Circotettix is almost entirely confined to the mountainous regions of western N. America, only a single species (*verruculatus* Kirby) being found in the mountains of the eastern U. S. A. (Maine, Massachussetts, New Hampshire, Connecticut, New York, New Jersey and Pennsylvania to Michigan). The members of this genus are essentially characteristic of rocky, forested regions over 5000 feet. Morphologically, they may be distinguished from species of Trimerotropis by the fact that certain of the veins of the hind wing are thickened; as a consequence of this the insects make a loud clattering sound with their wings while in flight.

According to the revision of REHN (1921) there are eight species of Circotettix, namely *undulatus* (Thomas), *verruculatus* Kirby, *thalassinus* Saussure, *shastanus* Bruner, *splendidus* Rehn and Hebard, *rabula* Rehn and Hebard, *coconino* Rehn and *crotalum* Rehn. The genus is closely related to Trimerotropis, and may be regarded as a montane derivative of it. Its affinities are clearly with section B of Trimerotropis rather than with section A, *T. suffusa* (formerly included by some authors in Circotettix) forming a connecting link.

Cytologically, the four species of Circotettix that have been investigated all present the same type of chromosome set as the members of section B of Trimerotropis, that is, they all have the X chromosome and some of the autosomes metacentric. They differ, however, from all species of Trimerotropis except *T. cyaneipennis* in having only 21 chromosomes in the male instead of 23.

The genus Aerochoreutes (REHN 1921) includes only a single species, *A. carlinianus* (Thomas) from British Columbia, Washington, Idaho, Montana, Wyoming, Nevada, Utah and Colorado. We have no personal experience of this species, but according to a statement by HELWIG (1942) it possesses metacentric chromosomes of the same type as those found in Circotettix and section B of Trimerotropis. It may have evolved from the same stock, although REHN (1921) has stated that it shows some affinities to the Old World genus Bryodema, whose cytology has not been studied.

The centromere shifts which are so characteristic of section B of Trimerotropis and of the genera Circotettix and Aerochoreutes may be defined as evolutionary conversions of acrocentric (rod-shaped) chromosomes into metacentric (V- or J-shaped) elements. This process has taken place without any reduction of chromosome number, that is, it is not a centric fusion (WHITE 1945). In an individual heterozygous for a centromere-shift there will be a pair of homologous chromosomes, one of which is a rod, the other a J or a V. Such a pair will obviously form an asymmetrical bivalent at meiosis. On the other hand if the individual is homozygous for a centromere-shift (that is, if it has a

pair of homologous V's), these latter will form a symmetrical 'metacentric bivalent' at meiosis.

There can be no doubt that the conversion has been from acrocentric to metacentric chromosomes (and not *vice-versa*) since the acrocentric type is the one almost universally found in the Acrididae, and especially in the subfamily Oedipodinae and the primitive section A of Trimerotropis. Theoretically, a centromere shift could occur in several different ways. We earlier assumed that the centromere shifts in this group of grasshoppers had probably arisen by pericentric inversions. If so, bivalents heterozygous for such a transposition should show a characteristic inversion-loop at pachytene. According to COLEMAN (1948), however, no loops can be seen in such bivalents at pachytene, pairing being complete from one end of the bivalent to the other, with no loops, unpaired regions or other peculiarities. This observation would seem to indicate that the centromere shifts of the Trimerotropis simply involved the transference of the centromere (perhaps with a minute region of the chromosome on either side) from a quasi-terminal to an interstitial position. Whether such a rearrangement involves two or three breakage points is uncertain; if MULLER's (1940) theory, according to which a chromosome end ('telomere') can never become interstitial, is correct, than a centromere-shift must necessarily involve three breakage-points. Otherwise, it is possible that only two breakage-points are necessary for such a rearrangement.

MATERIALS AND METHODS

The testes of male individuals were fixed in the field, either in San Felice's fixative or in acetic alcohol (1:3). Material fixed in the former fluid was later sectioned at 24 micra and stained by Newton's method. The testes fixed in acetic alcohol, on the other hand, were stained in bulk by the Feulgen technique, the individual follicles being then dissected out on a slide in 50 percent acetic acid and the lower part of each follicle, containing only sperm, being removed and discarded. The upper parts of the follicles were then lightly squashed under a cover-glass which was subesquently soaked off in acetic alcohol, the preparation being finally mounted in diaphane.

These methods are very different from those employed by the earlier workers who used Iron Alum Haematoxylin and were consequently obliged to cut their sections at 7–12 micra. These investigators rarely, if ever, were able to study whole nuclei, their illustrations being composite drawings built up from observation of two or even three successive sections. It will be obvious that the more modern techniques, using dyes that do not stain the cytoplasm appreciably and which consequently premit much thicker preparations (sections or squashes) to be used, are not only far quicker but also far less likely to lead to errors of interpretation.

The text figures illustrating the present paper are camera-lucida drawings reproduced at a uniform magnification of ×1860. We have taken the liberty of altering the relative positions of the chromosomes in drawing them, where overlapping of two or more chromosomes would lead to difficulties in interpretation if they were shown in their natural positions.

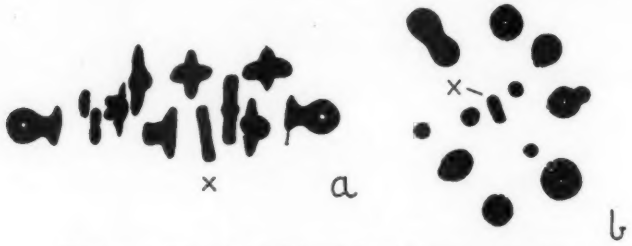
OBSERVATIONS

T. maritima Harris

We carried out a brief cytological examination of this species some years ago and found that it belongs to section A of the genus Trimerotropis, that is, all the 23 chromosomes in the male are acrocentric. We found no unequal bivalents or supernumerary chromosomes or any other cytological features of interest in five individuals studied.

T. citrina Scudder

A large population of this species was found along the sandy shores of the Pecos River, in the vicinity of Santa Rosa, N. M. (June 30). This is probably



TEXT-FIG. 1. *Trimerotropis citrina:* first metaphases. *a*, polar view; *b*, side view.

on the extreme western limit of the species, since HEBARD (1929) has shown that records from Colorado are erroneously based on material of *T. campestes*.

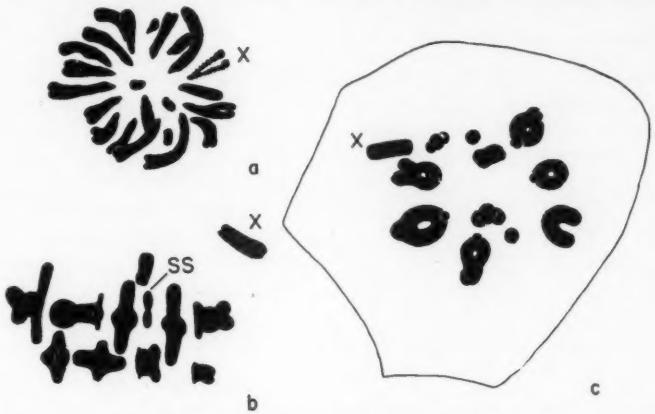
As is evident from the work of CAROTHERS (1931) this species belongs to section A of Trimerotropis—that is, it has all the chromosomes acrocentric. The three individuals studied by us did not show any cytological features of interest. The chiasma frequency is rather high for a Trimerotropis, as can be seen from figure 1. (the two largest bivalents frequently show three chiasmata each).

T. bilobata Rehn and Hebard

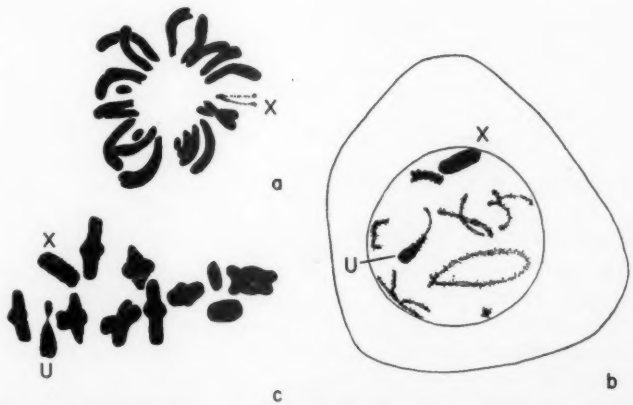
Three males and a single female of this species were collected in the sagebrush desert at a point about five miles northwest of Las Vegas, Nevada, on June 14, 1948.

As shown in figures 2 and 3, this species is a member of section A of the genus, all the chromosomes, including the X being acrocentric. Of the three males one possesses an unequal pair of chromosomes, while the other two are homozygous for the smaller member of this pair. In the homozygous individuals (fig. 2) the spermatogonial metaphases show three small chromosomes while in the heterozygous individual (fig. 3) there are only four small chromosomes, the homolog of one of them being one of the medium-sized chromosomes. In diplotene and diakinesis the unequal bivalent is very evident (fig. 3b) and one

can see that the 'extra' region which is present in the larger member of the pair is heterochromatic. At first metaphase the unequal bivalent shows up very clearly, the smaller chromosome being directed toward one pole, the larger one toward the opposite pole. Apparently a single chiasma is regularly formed in



TEXT-FIG. 2. *Trimerotropis bilobata:* cytologically homozygous individuals. *a*, spermatogonial metaphase; *b*, first metaphase in side view; *c*, in polar view. SS, the smallest bivalent (homozygous).



TEXT-FIG. 3. *Trimerotropis bilobata:* the individual with an unequal bivalent (U). *a*, spermatogonial metaphase (note that there are only 3 small chromosomes); *b*, diplotene (not all chromosomes shown); *c*, first metaphase in side view.

this bivalent (and never more than one). From a study of the small number of first anaphases and second divisions which occurred in the material it seems probable that the large and small members of this chromosome pair always separate at the first anaphase, and never at the second. We believe that the

'extra' region in the large member is proximal (that is, next to the centromere or including it) and that the chiasma is always at the distal end of the bivalent.

We hope to carry out more extensive work on the distribution of the long and the short types of this chromosome in natural populations of *T. bilobata*, a species which is widespread in the Great Basin.

T. latifasciata Scudder

Considerable doubt exists as to the specific distinctness of *T. latifasciata* Scudder (1880) and *T. laticincta* Saussure (1884). Provisionally, we shall consider them as synonymous, although TINCKHAM (1947) has followed MCNEILL (1901) in regarding them as distinct. The name *latifasciata* has been applied to specimens from the Great Basin area in which the band on the hind wing is narrower than in individuals from the Upper Sonoran zone of New Mexico and Arizona, heretofore called *lacticincta*. It is possible that the two forms are connected by a complete series of intermediates, constituting a cline, or they may prove to be "sibling species." HEBARD (1929) treats them, somewhat doubtfully, as distinct species. *T. melanoptera* NcNeill belongs to the same group of species and has an even broader band on the wing than *laticincta*.

TABLE 1

Trimerotropis latifasciata

Numbers of chromosomes in second meiotic divisions of individuals with one supernumerary chromosome.

INDIVIDUAL NO.	11 CHROMOSOMES (THAT IS WITHOUT EITHER X OR SUPERNUMERARY)	12 CHROMOSOMES (THAT IS WITH EITHER X OR SUPERNUMERARY	13 CHROMOSOMES (THAT IS WITH BOTH X AND SUPERNUMERARY)
777 H	10	20	7
777 L	17	25	20
Total	27	45	27

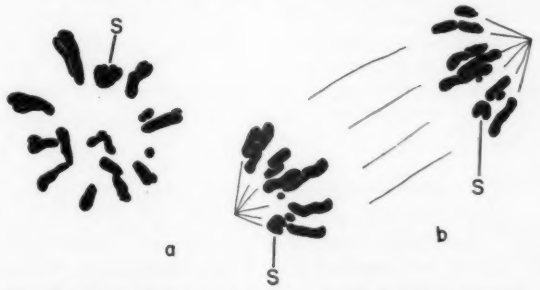
T. snowi Rehn 1905 is a strict synonym of *latifasciata*.

On July 1, 1948 we found a very dense population of *latifasciata* at Bottomless Lakes State Park, just E. of Roswell, N. M. The insects were extremely active and hard to catch, only 15 males being secured in about an hour's collecting. The rather local Truxaline *Pedioscirtetes maculipennis* was also common at this locality.

This proves to be one of those species of Trimerotropis in which all the chromosomes, including the X, are rod-shaped (acrocentric). It accordingly falls into section A of the genus. Of the 15 males two possessed a supernumerary chromosome, having 24 elements instead of 23 in the mitotic set. The extra element (fig. 4a) is a metacentric chromosome with arms of exactly (or almost exactly) equal length. In the two individuals with a supernumerary, this was present in every spermatogonial metaphase or anaphase examined, and in numerous cysts of spermatocytes in diplotene, diakinesis or first metaphase.

The supernumerary is thus not like those of Camnula (CARROLL 1920) which may be present in some cells and absent in others in the same individual; its mitotic behavior must be quite regular. At the first meiotic division it always seems to pass undivided to one pole with the other chromosomes, with-

TEXT-FIG. 4. *Trimerotropis latifasciata: a*, spermatogonial metaphase from an individual with a supernumerary chromosome (S); *b*, a first metaphase, in polar view, from an individual without any supernumerary; *c*, a first metaphase, in side view, from an individual with a supernumerary.



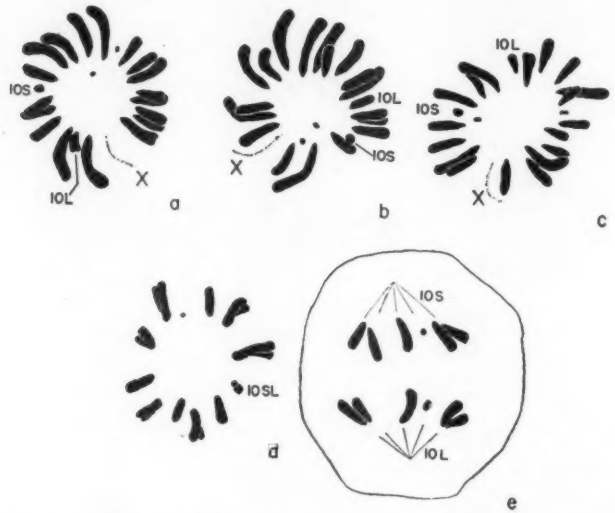
TEXT-FIG. 5. *Trimerotropis latifasciata: a*, second metaphase, showing the supernumerary (S); *b*, second anaphase, showing the division of the supernumerary.

out lagging on the spindle. None of the numerous anaphases and telophases of the first division which were studied suggested that the supernumerary was ever lost during the first division. A study of 99 second meiotic divisions in this material (table 1) is also quite compatible with the view that the supernumerary never divides and is never lost in the first division. Whenever it can be recognized as a metacentric element in second divisions (fig. 5a) one can always

see that it is split in preparation for the second anaphase, at which time it regularly divides.

T. sp. (= *T. titusi of* TINCKHAM, *not of* CAUDELL).

Two males and a female of a small Trimerotropis with distinctly-banded tegmina and yellow hind tibiae were collected June 24 at the entrance to Hualpai Mountain Park, near Kingman, Arizona. They agree in every respect with



TEXT-FIG. 6. *Trimerotropis sp.* (*titusi* of Tinckham): *a–c*, spermatogonial metaphases from individuals in which the 10th pair of chromosomes consists of two elements of unequal size (10 S and 10 L); *d*, a second-metaphase in polar view, with one element made up of a 10 S chromatid and a 10 L one, *e*, a second anaphase in side view, with 10 S and 10 L separating from one another (not all chromosomes shown).

TINCKHAM'S (1947) description of a form which he collected at the same locality and also at a locality north of Prescott, Arizona. TINCKHAM identified his material as *T. titusi* Caudell, a species from the coastal regions of California, but a comparison of our material with paratypes of *titusi* at the Academy of Natural Sciences, Philadelphia, makes it clear that this determination was erroneous. At the present time we are quite unable to identify the Hualpai form; it may constitute a new species or be a rather distinct geographical race of some already known species.

As shown in figure 6, this species definitely belongs to section A of the genus Trimerotropis, since all the chromosomes are acrocentric. A study of the spermatogonial metaphases revealed that in both individuals there were only three (instead of the expected four) small chromosomes. Two of these, which are minute, obviously constitute a pair, but the third, which is considerably larger,

has no obvious mate. It is only when we come to study the meiotic divisions that the position becomes clear: both individuals have an unequal bivalent of the type met with in individual No. 724 of *T. bilobata*. Apparently, however, the chiasma which is formed in this bivalent occurs between the centromere and the 'supernumerary segment' of the larger homolog (which we call 10 L, in contradistinction to the shorter element 10 S). Thus the segregation of the 10 L and 10 S chromatids from one another does not take place until the second anaphase (fig. 6 e). The behavior of this pair of chromosomes is thus exactly like that of the similarly unequal pair described by CAROTHERS (1931) in 11 out of 71 individuals of *T. citrina* (see her figures 2, 3, and 10).

T. pallidipennis pallidipennis Burmeister

This species has a wider distribution than any other member of the genus Trimerotropis. It ranges over almost the whole of western N. America, from Texas to California and from British Columbia and Alberta to the state of Oaxaca, at the extreme southern end of the Mexican Central Plateau. In Central America it is completely absent, but it reappears in Ecuador and is widely distributed in South America as far south as Patagonia (REHN 1939). Essentially a species of arid regions, it appears to thrive in a great variety of habitats and is frequently one of the commonest grasshoppers met with in semi-desert regions. In the earlier literature this species is referred to as *T. vinculata* Scudder.

Our material of *pallidipennis* was obtained from the following localities:

(1) 25 miles N. of Van Horn, Culberson Co., Texas (3 males studied).
(2) San Augustine Pass, Organ Mts. Doña Ana Co., N. M. (15 males studied).
(3) Pecos River near Santa Rosa, N. M. (3 males studied).
(4) 12 miles E. of Shiprock, N. M. (1 male studied).
(5) 21 miles North of Abiquiu, N. M. (8 males studied).
(6) Chiricahua Mountain Pass ,Cochise Co., Arizona (2 males studied).
(7) 16 miles North of Bridgeport, Ariz. (6 males studied).
(8) 12 miles N. E. of Payson, Ariz. (10 males studied).
(9) Kingman, Ariz. (1 male studied).
(10) Mormon Mesa N. E. of Moapa, Nevada (1 male studied).
(11) 46 miles N. W. of Las Vegas, Nevada (5 males studied).

Cytologically, *pallidipennis* proves to be a member of section B of Trimerotropis, since it has the X chromosome and three pairs of autosomes always metacentric. This is the condition invariably found in all the males studied by us. On the other hand, COLEMAN (1948) reports that 2 out of 16 males studied by him (from Vancouver Island) had only two metacentric bivalents. If this report is reliable it would seem to indicate an interesting difference between the populations of the species at the extreme northern end of its range and those found further south.

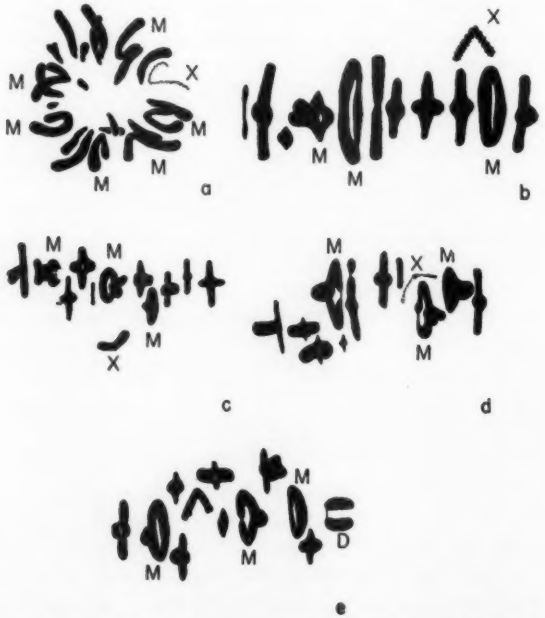
It will be seen from table 2 that in the overwhelming majority of the first spermatocytes all three metacentric bivalents form chiasmata in both arms.

TABLE 2

*Chiasmata in the three metacentric bivalents of T. pallidipennis**

INDIVIDUAL NO.	No. of Cells Having: 3 BIVALENTS WITH CHIASMATA IN BOTH ARMS	2 BIVALENTS WITH CHIASMATA IN BOTH ARMS	1 BIVALENT WITH CHIASMATA IN BOTH ARMS	0 BIVALENT WITH CHIASMATA IN BOTH ARMS
695 E	16	4	–	–
695 F	13	1	2	–
695 G	16	3	1	–
695 S	12	1	–	–
754 B	18	4	–	1
754 C	18	2	–	–
Total	93	15	3	1

* The actual number of chiasmata is not given in Tables 2–6; sometimes more than one is present in a chromosome arm, but this cannot always be determined with certainty at metaphase.



TEXT-FIG. 7. *Trimerotropis pallidipennis: a*, spermatogonial metaphase, with the six metacentric autosomes labelled M; *b–e*, first metaphases in side view, showing the three metacentric bivalents always present. *b* and *e* are from Feulgen squash preparations in which the chromosomes always appear larger than in sectioned material. In *e* one of the bivalents (D) shows a chiasma in the short arm and none in the long arm.

These bivalents form from one to three chiasmata, ring-bivalents with two chiasmata being the most common type. The acrocentric bivalents mostly have only one chiasma, occasionally two in the larger bivalents.

There is one autosomal bivalent in *pallidipennis* which quite frequently forms a single chiasma in the *short arm* (element D in fig. 7e). Occasionally it may form one chiasma in the short arm and one in the long arm, but this is exceptional. In a single first metaphase in one individual (No. 768E from 21 miles north of Abiquiu, N. M.) this element was represented by two univalents; obviously, either pairing or chiasma formation had failed to occur.

T. cyaneipennis Bruner

Two males of this species were studied cytologically. The first was collected

TEXT-FIG. 8. *Trimerotropis cyaneipennis: a*, first metaphase, side view, from individual No. 785; *b* and *c*, first metaphases from individual No. 730. M, metacentric bivalents; in *a* two of the eight metacentric bivalents have chiasmata in both arms, in *b* four of the six do so, in *c* five metacentric bivalents have chiasmata in both arms.

in a box canyon on Stonewall Mountain, approximately 18 miles southeast of Goldfield, Nevada (June 15), the second at Caliente, Nevada (June 20).

As determined by KING (1924) this species of Trimerotropis is apparently unique in having only ten pairs of autosomes, that is, the spermatogonia contain 21 instead of the usual 23 chromosomes. The first individual (No. 730) was homozygous for six pairs of metacentric chromosomes, the second one (No. 785) was homozygous for eight such pairs. In both the X was metacentric, as in other members of Section B. Neither individual possessed supernumerary chromosomes or was in any way cytologically heterozygous.

KING (1924) studied seven individuals of this species from the Grand Canyon. Most of these seem to have been heterozygous for one centromere-shift, but the potential heterozygosity was greater than this, since the number of meta-

TABLE 3

Chiasmata in the metacentric bivalents of T. cyaneipennis

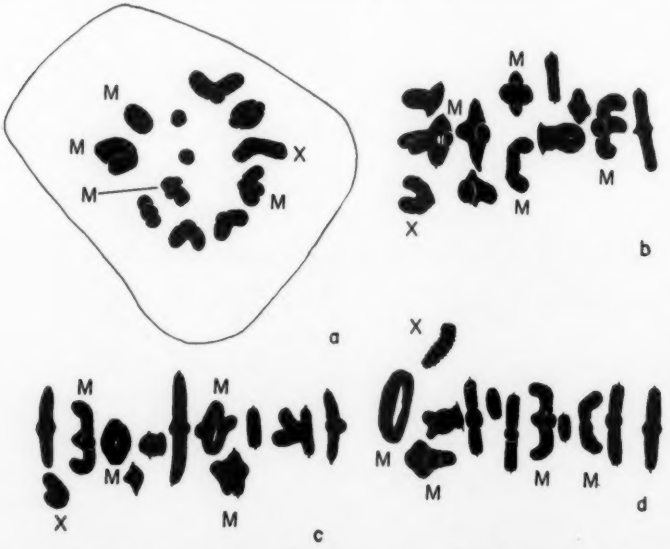
INDIVIDUAL NO.	No. of Cells Having: 5 bivalents with chiasmata in both arms	4 bivalents with chiasmata in both arms	3 bivalents with chiasmata in both arms	2 bivalents with chiasmata in both arms
730 (6 metacentric bivalents)	4	3	–	–
785 (8 metacentric bivalents)	1	3	3	8

centric chromosomes ranged from 13 (as in our No. 730) to 17 (as in our No. 785). One of KING's individuals possessed a supernumerary chromosome.

Some data on the distribution of the chiasmata in the metacentric bivalents of our two individuals are given in table 3. It will be noted that most of the metacentric bivalents form chiasmata in one arm only, which is not unexpected if these bivalents are liable to be heterozygous in some individuals.

T. suffusa Scudder

This is a highly variable species which may be separable into several geographical races. HEBARD (1929) concluded that *T. fallax* Saussure was at best



TEXT-FIG. 9. *Trimerotropis suffusa: a*, first metaphase in polar view; *b–d*, first metaphases in side view.

only a western subspecies of *suffusa*, but the exact status of the forms included in this group is still uncertain.

So far, we have only studied a single individual of *T. suffusa*, from a locality 21 miles north of Abiquiu, N. M. (June 29). This grasshopper was homozygous for four metacentric chromosomes, and in addition possessed a metacentric X, like all the species of section B. Although spermatogonial metaphases were not found in the slides, they must have contained nine metacentric elements and fourteen acrocentrics. This is very close to the average of 8.9 metacentrics determined by CAROTHERS (1917) for material of this species (her 'form B'). No supernumerary chromosomes were present in the Abiquiu individual, although they were found in some individuals of this species by CAROTHERS and COLEMAN. We give, in table 4 some data on the distribution of chiasmata in the four metacentric bivalents of the Abiquiu individual.

TABLE 4

Chiasmata in the four metacentric bivalents of T. suffusa (individual No. 769)

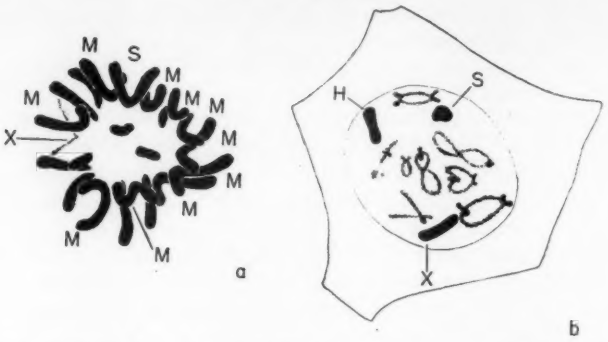
No. of Cells Having:			
4 bivalents with chiasmata in both arms	3 bivalents with chiasmata in both arms	2 bivalents with chiasmata in both arms	1 bivalents with chiasmata in both arms
—	4	33	13

C. undulatus Thomas

A single individual of this species was collected at Kingston Canyon, just below Toiyabe Range Peak, Nye Co., Nevada. Like other species of the genus, this one proves to have only ten pairs of autosomes.

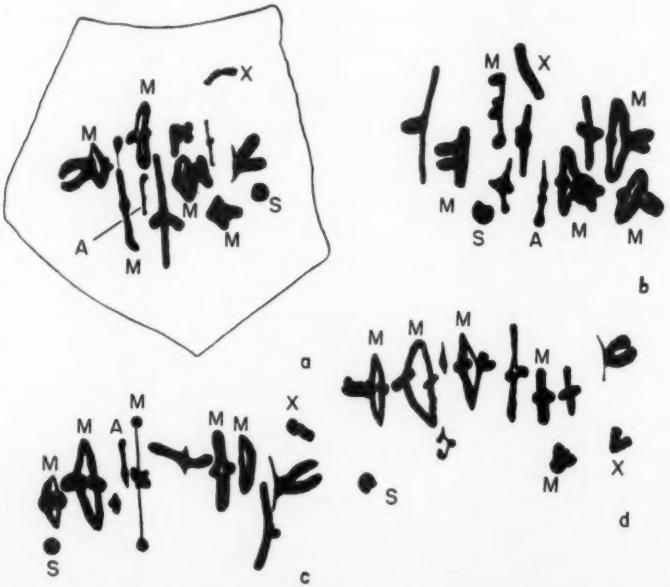
The individual studied possesses a supernumerary chromosome which like those of *T. latifasciata*, is a metacentric element with arms of equal (or almost equal) length, as can clearly be seen in spermatogonial metaphases; it is not difficult to distinguish the supernumerary from the metacentric autosomes, since the latter all have arms of distinctly unequal lengths (fig. 10 a). During the prophase of the first meiotic division the nuclei show three heteropycnotic bodies (fig. 10 b). One of these is regularly sausage-shaped and represents the X, the second is a heteropycnotic autosomal bivalent and the third is the supernumerary, very much condensed and appearing almost spherical or slightly bilobed or kidney-shaped.

The behavior of the supernumerary chromosome at the anaphase of the first meiotic division differs from that of the supernumerary in *T. latifasciata* and is somewhat variable. It nearly always lags behind the other chromosomes in the spindle (fig. 12 a and b). In the majority of cases it seems to pass undivided to one of the two daughter cells, appearing as a four-armed body, which may be included in the main interkinetic nucleus but which more frequently forms a small supplementary micronucleus adjacent to the main nucleus. In some instances, however, the supernumerary divides in the late anaphase of the first division, in which case its two halves always seem to pass to opposite poles, so



TEXT-FIG. 10. *Circotettix undulatus:* an individual with a supernumerary chromosome. *a*, spermatogonial metaphase, showing ten ordinary metacentric autosomes and the supernumerary (S); *b*, diplotene, showing the three heteropycnotic elements, the X, the supernumerary and a heteropycnotic autosomal bivalent.

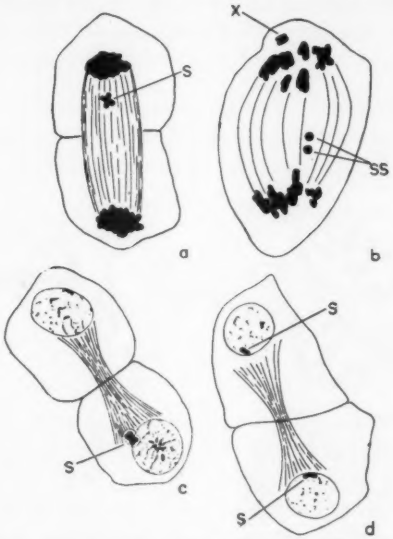
that each daughter cell receives a supernumerary element. The limited material available (a single cyst of first anaphases, telophases and interkinetic cells) did not provide any evidence of loss of supernumerary chromosomes during the first division, nor were any instances of the two daughter super-



TEXT-FIG. 11. *Circotettix undulatus:* first metaphases. S, the supernumerary chromosome, M., the metacentric bivalents. A, the small asymmetrical bivalent (not recognizable in *d*).

numeraries passing to the same pole seen. There were no cysts of second meiotic divisions in the material available, but it is reasonable to suppose that those supernumeraries which have divided in the first division do not do so in the second one, and that those which have not divided in the first division do so in the second. If so, an individual with a single supernumerary (such as the particular specimen studied) would produce equal numbers of sperms with and without the supernumerary.

The supernumerary shows marked positive heteropycnosis during inter-



TEXT-FIG. 12. *Circotettix undulatus:* first anaphases and telophases.

kinesis, that is, it remains condensed and dark-staining long after the other chromosomes, including even the X, have become de-condensed and weak-staining.

The present individual possessed five pairs of large metacentric chromosomes. At meiosis all of these may form chiasmata in both arms, but they do not always do so (table 5). In addition one of the two smallest bivalents is

TABLE 5

Circotettix undulatus—Individual No. 783 (with 5 metacentric bivalents)

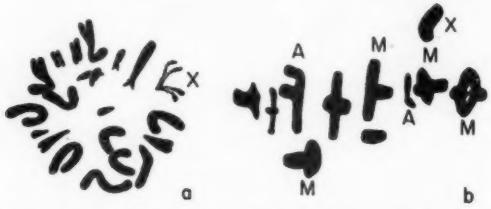
NO. OF CELLS HAVING:		
5 BIVALENTS WITH CHIASMATA IN BOTH ARMS	4 BIVALENTS WITH CHIASMATA IN BOTH ARMS	3 BIVALENTS WITH CHIASMATA IN BOTH ARMS
8	21	8

slightly asymmetrical (fig. 11 a, b, c). Apparently this little bivalent is made up of two chromosomes whose centromeres are not in the same locus, but a detailed study would be very difficult; in some cells the asymmetrical nature of the bivalent is not clear—this may depend on the position of the chiasma.

This species is identical with *C. lobatus* Saussure, under which name CAROTHERS (1917) described the cytology of eleven individuals from La Grande, Ore. In several of these individuals the smallest bivalent was heterozygous for a centromere-shift, as in our specimen, but in addition, several of CAROTHERS' individuals were heterozygous for such shifts in the larger chromosomes, although three out of the eleven were cytologically homozygous. Two of CAROTHERS' individuals possessed a supernumerary chromosome.

C. rabula altior Rehn

A single individual of this form was collected at a locality 21 miles north of Abiquiu, N. M. (June 29). Like other species of the genus it proves to have 21



TEXT-FIG. 13. *Circotettix rabula altior: a*, spermatogonial metaphase; *b*, first metaphase in side view. A, the asymmetrical bivalents.

chromosomes in the male (instead of 23, the usual number in the Acrididae). No supernumerary chromosome was present. As seen in the spermatogonial divisions (fig. 13 a) there were ten large metacentric chromosomes, one of which is the negatively heteropycnotic X chromosome which is clearly divided into its two chromatids at metaphase. There are three pairs of large or medium-sized acrocentric (rod-shaped) elements and two pairs of small chromosomes which usually appear as rods, but whose exact shape is not entirely clear in the spermatogonial metaphases.

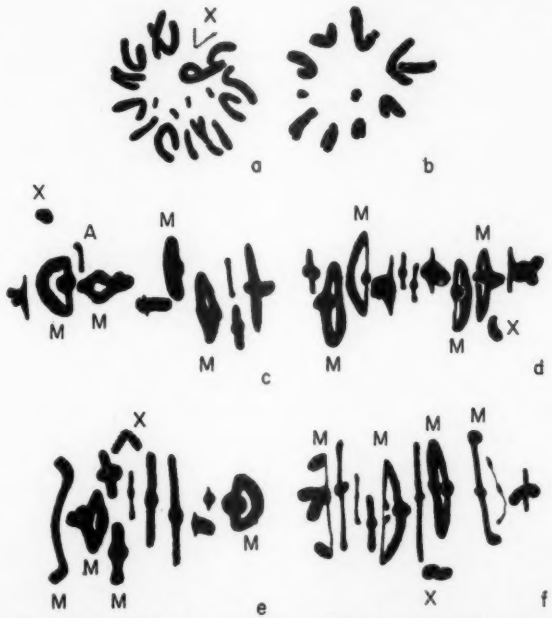
The first meiotic divisions in this individual show the X, four metacentric bivalents, four acrocentric bivalents and two asymmetrical bivalents of which one is quite large, the other much smaller (fig. 13 b). The metacentric bivalents almost always have chiasmata in both arms and sometimes one arm contains two chiasmata. The asymmetrical bivalents, on the other hand, have a much lower chiasma-frequency, since they apparently never form chiasmata in the region between the centromere of one homolog and that of the other. There is thus no reason to suppose that this individual produced any dicentric or acentric chromosome strands at meiosis, although the slides did not contain a sufficient number of first anaphases for a direct check on this point.

It is of course, not legitimate to speculate as to the genetic structure of a species from a cytological examination of a single individual, but certain con-

clusions seem to be justified. It is clear, for example, that *C. rabula altior* is a species in which heterozygosity for centric shifts occurs (the particular individual was heterozygous for two such). It also appears probable that the bivalents which show such heterozygosity have a type of chiasma-localization which prevents them from forming large numbers of inviable gametes (that is, ones with broken chromosomes or with acentric ones). Whether these bivalents show the same type of localization when homozygous is uncertain. We hope to obtain further data on this interesting species next year. The only previous record of cytological work on this species is by CAROTHERS (1917, p. 464) who studied a single individual from Ogden, Utah.

C. crotalum Rehn

This species of Circotettix, described by REHN (1921) has an extremely restricted distribution, being confined, so far as is known, to certain mountains



TEXT-FIG. 14. *Circotettix crotalum: a*, spermatogonial metaphase; *c*, second metaphase, *c–f*, first metaphases in side view. Abbreviations as in previous figures.

in southern Nevada, where it occurs from 8,000 to 10,200 feet in rocky limestone environments where pinyon, yellow pine and juniper are the dominant trees. The original series collected by REHN and HEBARD was obtained at two localities (Lees Canyon and Charleston Peak) in the Spring Mountains. Our material (49 males and a number of females) was collected June 22–23 at

Hidden Forest, a canyon below Sheep Peak, approximately 35 miles northeast of REHN and HEBARD's localities and 30 miles north of Las Vegas. In this canyon, which is fairly thickly wooded, although surrounded on all sides by the yucca-dotted sagebrush desert, the species was extremely common, hardly any other Acrididae except a single individual of *Trimerotropis cyaneipennis* being seen. At this date the population of *C. crotalum* consisted of nymphs and newly-metamorphosed imagines in approximately equal numbers.

The Hidden Forest population of *C. crotalum* is very completely isolated, geographically. It is possible that the species occurs in other parts of the Sheep Range and in the Las Vegas and Desert Ranges which run parallel to it. But

TABLE 6

Chiasmata in the four metacentric bivalents of C. crotalum

	No of Cells Having:			
INDIVIDUAL NO.	4 BIVALENTS WITH CHIASMATA IN BOTH ARMS	3 BIVALENTS WITH CHIASMATA IN BOTH ARMS	2 BIVALENTS WITH CHIASMATA IN BOTH ARMS	1 BIVALENT WITH CHIASMATA IN BOTH ARMS
737 C	2	4	2	–
738 C	4	–	–	–
738 D	2	3	–	2
739 A	8	2	1	–
739 B	6	4	2	1
739 C	7	4	–	–
739 D	4	1	1	–
Total	33	18	6	3

all these ranges are separated by wide desert valleys which would seem to constitute formidable barriers to a species characteristic of pine forests. The species is thus one in which we might expect to be able to observe the effects of long isolation of the individual populations from one another, but the particular population studied was sufficiently large (several thousands of individuals at least) for the frequency of the various chromosomal types to be determined mainly by natural selection rather than by "drift."

C. crotalum, or at any rate this population of it, proves to be relatively uniform, cytologically. The X and four large pairs of autosomes are metacentric in all individuals studied. Five pairs of autosomes are always acrocentric, but one of the two smallest autosomes may be either acrocentric or metacentric, so that some individuals have one small asymmetrical bivalent. This, however, is extremely difficult to score consistently, so that it is unsuitable for statistical study. One of the four pairs of metacentric chromosomes has the two arms of almost exactly equal length, the other three have unequal arms ("J-shaped" chromosomes). At the first meiotic division it is usual for all the four metacentric bivalents to form chiasmata in both arms; but in some cells one, two or three of the metacentric bivalents have chiasmata in one arm only (see table

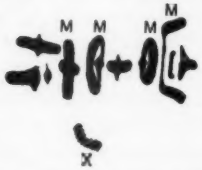
6). It is clear, however, that there is little chiasma-localization in the metacentric bivalents of this species.

The Hidden Forest population of *C. crotalum* does not appear to possess any supernumerary chromosomes—at least none were found in the 21 individuals studied cytologically. In this respect *crotalum* resembles *C. verruculatus* (HELWIG 1929) and differs from *C. undulatus* (=*lobatus*) in which both we and CAROTHERS (1917) have found supernumerary elements. In the case of *C. rabula* only two individuals have been studied cytologically (one by CAROTHERS, one by ourselves), so that it is uncertain whether supernumeraries exist or not.

C. crotalum is not completely homozygous, cytologically speaking, since one of the smallest chromosomes may be either acrocentric or metacentric; but it shows less heterozygosity than the other three members of the genus which have been studied, all of which may be heterozygous for centric shifts in several chromosomes. It is unfortunate that the centric shift in the small chromosome could not be studied in detail. We did find among the 21 individuals studied all three expected types—those homozygous for an acrocentric chromosome, those heterozygous, and those homozygous for a metacentric element —but these were so difficult to distinguish, on account of the small size of this bivalent, that we came to the conclusion that it would be dangerous to attempt to classify the individuals into the three types, since errors would undoubtedly be made.

C. coconino Rehn

A single male of this species, from Williams, Ariz., shows the general cytological characteristics of the genus, namely only ten pairs of autosomes and a



TEXT-FIG. 15. *Circotettix coconino:* first metaphase.

metacentric X. The four largest pairs of autosomes are metacentric, as in *C. crotalum*, no asymmetrical bivalents being present. There were no supernumerary chromosomes.

GENERAL DISCUSSION

Natural populations of grasshoppers belonging to the genera Trimerotropis and Circotettix seem to show four different types of cytological polymorphism:

(1) *Supernumerary chromosomes*, present in some individuals but not in others.

(2) *Supernumerary chromosome regions*, present in some individuals but not in others (leading to the presence of unequal bivalents when heterozygous).

(3) *Centromere-shifts* may be present in some individuals but not in others (leading to presence of asymmetrical bivalents when heterozygous).

(4) *Translocations* may be present in some individuals but not in others (leading to rings or chains of four chromosomes at meiosis when heterozygous).

For the most part, each species only shows one or two of these types of cytological variability. Thus translocations have only been found in *T. citrina* and *T. suffusa fallax* (HELWIG 1933), supernumerary chromosomes occur in *T. latifasciata*, *T. suffusa*, *T. cyaneipennis* and *C. undulatus* (but not so far as known, in the other species which have been studied); and centromere-shifts have not been recorded in any of the species belonging to section A of Trimerotropis.

Supernumerary chromosomes in grasshopper populations seem to be of two kinds: in the first place we have those studied by CARROLL (1920) in *Camnula pellucida*, which vary in number in different cells of the same individual, apparently because they undergo mitotic nondisjunction rather frequently. ROTHFELS (1948) has recently described an individual of *Neopodismopsis* in which several supernumerary elements were present, but here the number of supernumeraries varied from cyst to cyst (although constant within each cyst) In the second place we have supernumeraries of the type recorded by CAROTHERS for *T. suffusa* and studied by us in *T. latifasciata* and *C. undulatus*, which seem to be constant in number for all the cells of an individual (at least in the testis). This last type always seem to be metacentric elements (even in *T. latifasciata*, which has no other metacentrics), and their arms are always equal in length (or very nearly so). It is possible that they are isochromosomes, that is, that their two arms are homologous, but it is difficult to be certain of this. Always exhibiting positive heteropycnosis during the prophase of the first meiotic division, they show no heteropycnosis during the spermatogonial metaphases, when the X usually shows very pronounced negative heteropycnosis (in all those species of Trimerotropis and Circotettix which we have studied). Furthermore, the supernumeraries show no tendency to pair with the X at meiosis, so that there is no necessity to believe that they are in any way derived from it or homologous to any part of it.

Most of the discussions as to origin of supernumerary chromosomes which occur in the literature are somewhat unsatisfactory. Obviously, any supernumerary chromosome must have originated, either from some part of the chromosome complement of the species in which it occurs, or from another species, by hybridization (this may account for some supernumerary chromosomes in plants). The supernumeraries of *T. latifasciata* and *C. undulatus* are obviously not homologous to any part of the existing chromosome complement of those species, since they never pair at meiosis with any other chromosome. Does this mean that they were derived from some other species? We do not believe so, unless they have been handed down from ancestral species, without any hybridization having occurred. We believe that these elements were originally duplications of some part of the regular chromosome complement which have undergone an extensive series of rearrangements since their origin, thereby eliminating any structural homology to any member of the regular

complement. It is probable that these supernumeraries, when they first arose, showed mitotic instabilities of the type still shown by the supernumeraries of Camnula (CARROLL 1920) and Neopodismopsis (ROTHFELS in press). Such instabilities, due to an inefficient centromere-mechanism, may have led, on the one hand to frequent mitotic nondisjunction, and on the other to repeated deletions and duplications. The fact that the supernumeraries become contracted into an almost spherical shape during the prophase of meiosis suggests that they possess duplicated regions which exhibit some type of pairing. A study of individuals with two or more supernumeraries might help to elucidate this point. The fact that the supernumeraries of Trimerotropis spp. and Circotettix spp. are heterochromatic suggests that they are genetically semi-inert; but we feel sure that their widespread occurrence in some of these species (CAROTHERS 1917; WENRICH 1917) indicates that they are not entirely devoid of genetic properties. It is hardly profitable to speculate as to whether they were originally derived from the X chromosome or from autosomal heterochromatin. The fact that they do not show negative heteropycnosis during the spermatogonial divisions would seem, on the face of it, to indicate the latter: but it is by no means impossible that the X of these species (or their ancestors) contains (or contained) more than one type of heterochromatin, and that the supernumeraries were built up by repeated duplication of a small region in the X which did not show the typical "reversal of heteropycnosis" characteristic of the X as a whole (WHITE 1942).

Supernumerary chromosome regions, such as give rise to unequal bivalents in some individuals of *T. bilobata* and *T. sp.* have been previously recorded for many species of grasshoppers, for instance in *Brachystola magna*, *Arphia simplex* and *Dissosteira carolina* by CAROTHERS (1913).

Some confusion exists in the literature concerning the terminology to be applied in such cases. Thus some authors have referred to individuals with an unequal bivalent as heterozygous for a deficiency, others calling them heterozygotes for a duplication. These terms are derived from Drosophila genetics, and neither seems to be strictly applicable in such cases. An unequal bivalent consists of a short chromosome and a long one which contains a heterochromatic segment that is lacking in the short element. There is no reason to suppose that this heterochromatin represents a duplication of material present elsewhere in the chromosome complex, although it may do so. Thus the term duplication seems rather inappropriate. But to say that a chromosome lacking an inessential heterochromatic region is carrying a deficiency also seems confusing. These supernumerary chromosome regions in grasshoppers seem, in fact to be genetically similar to supernumerary chromosomes, but instead of constituting independent elements they are inserted into a member of the regular chromosome complement, usually one of the smaller ones.

Heterozygosity in respect of centromere shifts is obviously entirely different from heterozygosity for an extra chromosome region. At the time when we first reviewed the cytological situation in Trimerotropis and Circotettix (WHITE 1945) we considered the possibility that the 'asymmetrical bivalents' in some of the species might be due to pericentric inversions. Since that time, however, COLEMAN (1948) has shown that pachytene bivalents heterozygous

for these structural changes (that is, which will appear as asymmetrical bivalents at first metaphase) do not show any inversion-loops such as would be seen if pericentric inversions were present. On the contrary, pachytene pairing seems to be quite complete, the bivalents showing no unpaired regions. It is accordingly fairly clear that true centromere shifts, that is, transpositions of the centromere (possibly with a very short region on either side) are responsible for the asymmetrical bivalents.[2]

We have already considered (WHITE 1945) some of the consequences of heterozygosity in respect of a centromere shift. In such a bivalent a chiasma which occurs between the two centromeres will necessarily lead to the formation of a dicentric chromatid and an acentric one. Thus heterozygosity for centromere shifts would lead to the formation of a large percentage of inviable gametes unless the chiasmata were strictly localized at the other end of the chromosome, so that they were never formed between the two centromeres. A study of *C. rabula altior* and an inspection of the published figures of CAROTHERS and HELWIG suggests that such a localization does in fact occur in all those bivalents which are liable to be heterozygous in some individuals. We still do not have enough data, however, to determine whether in these cases chiasmata are *never* formed in the "prohibited" region, or whether they may occasionally occur there, leading to a few inviable gametes. Nor is it certain whether localization occurs in the female: it is conceivable that in oogenesis dicentric and acentric chromatids would be lost in the polar bodies.

Another unanswered question is whether localization of chiasmata or centromere shifts came first in evolution. In other words, did an already-existing localization permit centromere shifts to establish themselves in the wild populations of these grasshoppers, or did centromere shifts establish themselves first, leading to a high percentage of sterile gametes until natural selection gave rise to an increasing degree of chiasma-localization? All the species in which heterozygosity for centromere shifts occurs also show some bivalents which are homozygous for such shifts (and *T. pallidipennis* is homozygous for three shifts in the autosomes and shows no heterozygosity). But in the case of these bivalents which are homozygous for centromere shifts no chiasma localization is evident—or at any rate chiasmata are regularly formed in both arms of the bivalent. Thus in such cases the newly arisen metacentric type of chromosome would seem to have displaced the original acrocentric type in the natural populations of the species, in spite of the fact that the heterozygotes must have been relatively infertile (and of course in the initial stages of the process there would have been few if any individuals homozygous for the metacentric type of chromosome). A possible but entirely hypothetical explanation would be to suggest that in these cases chiasma localization was originally present, but that it disappeared after the original, acrocentric type of chromosome vanished from the populations. We still lack essential data bearing on this and related points: we do not even know, for example, whether the chiasma frequency of a particular chromosome is the same, irrespective of whether it is in an AA, AM or MM bivalent.

[2] CROUSE (1947) has reported the existence of a similar centromere shift which has occurred in the C chromosome of *Sciara ocellaris* in certain strains.

We have thought it worth while to give some data on the frequency of chiasma formation in the two arms of the metacentric bivalents (tables 2–6) since it seems likely that a bivalent which usually shows chiasma in both arms is *a priori* unlikely to show heterozygosity, while bivalents such as some of those of *T. cyaneipennis* and *T. suffusa* (tables 3 and 4), which normally show chiasmata only in one arm, may well be heterozygous in some individuals. In *C. crotalum* we conclude from table 6 that none of the four metacentric bivalents is likely to show heterozygosity in other populations of the species.

Genetically speaking, the effect of a centromere shift combined with chiasma localization may be rather similar to that of an inversion in Drosophila—that is, it may give rise to a group of linked genes which are inherited as a unit in heredity without being broken up by crossing over. And it may be that, just as in the case of *Drosophila pseudoobscura*, the heterozygous individuals have a higher viability than either of the homozygous types, thus leading to the perpetuation of both the acrocentric and metacentric types of chromosome. But much more work will need to be carried out before any such statement can be regarded as firmly founded in fact.

On the basis of the earlier work of CAROTHERS, WENRICH and KING and the more recent work of COLEMAN and ourselves we may now classify the species of Trimerotropis into the two sections A and B as follows:

Section A (All chromosomes acrocentric)	*Section B* (Some chromosomes, including always the X, metacentric)
T. maritima Harris (W)	
T. citrina Scudder (C, W)	*T. pallidipennis* Burm. (Co., W)
T. latifasciata Scudder (W)	*T. suffusa* Scudder (C, Wen., W)
T. bilobata Rehn and Hebard (W)	*T. cyaneipennis* Bruner (K, W)
T. sp. (=*titusi* of Tinckham) (W)	*T. caeruleipennis* Bruner (K)
T. fontana Thomas (Co.)	*T. thalassica* Bruner (K)
T. praeclara McNeill (Co.)	*T. gracilis sordida* Walker (Co.)

(C = CAROTHERS, Co. = COLEMAN, K = KING, Wen. = WENRICH, W = WHITE)

Of the species in section B, *pallidipennis* and *gracilis sordida* show relatively few metacentric chromosomes, the other four species much higher numbers. As far as our experience goes, *pallidipennis* seems to be a cytologically homozygous species in which three chromosome pairs are invariably metacentric, the remaining eight being always acrocentric. It is possible, however, that in some individuals of *pallidipennis* from Vancouver Island there are fewer metacentric chromosomes than in material from the Great Basin and the Southwest.

A full discussion of taxonomic relationships within the Trimerotropine grasshoppers would be premature at the present stage. Clearly, section A of Trimerotropis includes the more primitive species, in which none of the chromosomes have become metacentric. It is still uncertain, however, whether section B represents a strictly monophyletic group, descended from a single species in which centromere shifts established themselves in the X and in several autosomes. The alternative would be to suppose that the species of section B do not form

a monophyletic group, metacentric chromosomes having appeared in several ancestral species, not necessarily closely related to one another. It is virtually certain that Circotettix arose from section B of Trimerotropis.

SUMMARY

The genus Trimerotropis may be divided, on cytological grounds, into two sections. In the first of these all the chromosomes are acrocentric (rod-shaped), while in the second a varying number of elements (including always the X chromosome) have been converted into metacentric chromosomes by shifts of the centromere region from a subterminal to a submedian position. All species of the related genus Circotettix have chromosome complements of the second type, that is, with a certain number of metacentric chromosomes.

No chromosomal variation was encountered in small samples of *T. maritima* and *T. citrina*, which belong to the first section of Trimerotropis. In *T. latifasciata* a large metacentric supernumerary chromosome is present in about one seventh of the individuals of a population at Roswell, N. M. A similar supernumerary was also found in a single individual of *Circotettix undulatus*. In a population of *T. bilobata* from Las Vegas, Nevada, and in an unidentified species of Trimerotropis from near Kingman, Arizona unequal pairs of chromosomes (due to the presence of a supernumerary region in one homolog) were found in some individuals.

In *Circotettix crotalum*, *C. undulatus* and *C. rabula altior* heterozygosity in respect of the position of the centromere occurs in some bivalents of some individuals. It is pointed out that this condition must be accompanied by a special type of chiasma localization if it is not to lead to the formation of a large number of inviable or lethal gametes. In *Trimerotropis pallidipennis* 55 individuals from Texas, New Mexico, Arizona and Nevada showed no cytological variation or heterozygosity.

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A STUDY OF TRYPTOPHANE IN EYE COLOR MUTANTS OF DROSOPHILA[1]

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EARLIER studies have yielded evidence which points to the amino acid tryptophane as a precursor of the brown eye pigment in adult Drosophilae as well as in other insects. The precise mechanism whereby tryptophane is converted to brown pigment has been in part clarified as a result of a number of investigations. In the case of *Drosophila melanogaster* these investigations have been primarily concerned with the mutants vermilion and cinnabar, in which the phenotype is characterized by a lack of brown eye pigment. The transplantation studies of BEADLE and EPHRUSSI (cf. EPHRUSSI 1942 for a summary) confirmed earlier observations of STURTEVANT (1932) that genetically vermilion eye discs develop non-autonomously in a wild type host, that is, they form brown pigment. They suggested that the failure of brown pigment formation in vermilion flies was related to the absence of a substance designated the v^+ hormone. Further study demonstrated the non-autonomous behavior of cinnabar eye discs when transplanted into wild type hosts and led to the suggestion that a second substance, designated the cn^+ hormone, was necessary for the development of brown pigment.

The nature of the v^+ hormone was ultimately determined from the results of a number of investigations. In the course of nutritional studies on the vermilion gene of *D. melanogaster*, TATUM (1939) observed that when vermilion flies were raised on a tryptophane-containing medium which had been contaminated by an unknown bacillus, brown pigment was laid down in the adult eyes. BUTENANDT, WEIDEL, and BECKER (1940) then showed that the tryptophane metabolite kynurenin derived from rabbit urine, when injected into or fed to developing vermilion flies, resulted in the deposition of brown pigment in the adult eyes. The bacterial substance was then demonstrated to be kynurenin (TATUM and HAAGEN-SMIT 1941). Final proof of the identity of the v^+ hormone with kynurenin was offered by KIKKAWA (1941), who isolated the kynurenin from cinnabar pupae of *D. melanogaster*.

It has been proposed by a number of investigators that in the formation of brown eye pigment, tryptophane is metabolized in the following manner:

tryptophane→α oxytryptophane→kynurenin→cn^+ hormone—brown pigment

KIKKAWA has suggested that the cn^+ substance is the chromogen of the brown pigment. However, at the present time the nature of the cn^+ hormone, and the intervening steps to brown pigment formation, are unknown.

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The present investigation was undertaken in an attempt to determine the fate of tryptophane in various eye color mutants of *D. melanogaster* and *D. virilis*. In the case of the vermilion mutant of Drosophila, where the lack of brown pigment is associated with a failure in kynurenin formation, it is of interest to learn the reason for this failure in the oxidation of tryptophane. One explanation offered is that an insufficient quantity of non-protein tryptophane is available to developing vermilion flies with a resultant absence of kynurenin. CASPARI (1946), studying the recessive *a* mutation in Ephestia which is similarly characterized by a failure in the formation of kynurenin, observed a significant increase in the protein tryptophane in the mutant as compared with the normal (a^+) moths. He suggested that the increased protein tryptophane might in part account for the failure in kynurenin formation by limiting the non-protein tryptophane available for oxidation. A second possibility is that in vermilion, the tryptophane is metabolized along an entirely different pathway which excludes the formation of kynurenin. Such an explanation has been suggested by BEADLE and TATUM (1941), who pointed out that the tryptophane might be oxidized to kynurenic acid in place of kynurenin. A third possible explanation is that the vermilion flies possess sufficient non-protein tryptophane for conversion to kynurenin but lack the enzyme(s) which are capable of catalyzing this oxidation. If this were the case, one would expect an accumulation of non-protein tryptophane.

In the case of the other eye color mutants, where it is known that tryptophane is oxidized to kynurenin, it is of interest to know how much of the tryptophane is converted to kynurenin; and especially in the case of the *cn* mutant, what the fate of kynurenin is in these flies.

To select the most satisfactory explanation, a number of tryptophane analyses as well as estimates of kynurenin have been made on certain eye color mutants of *D. melanogaster* and *D. virilis*. A preliminary report of these results has been made (GREEN 1948).

MATERIALS AND METHODS

The following homozygous mutants of *Drosophila melanogaster* were investigated: vermilion (*v*); cinnabar (*cn*); scarlet (*st*); brown (*bw*); suppressor of vermilion in combination with vermilion (su^2-*s*, *v*); and white (*w*). A Stephenville wild type strain served as the wild type control. In *Drosophila virilis* the following homozygous mutants were investigated: vermilion (*v*); cinnabar (*cn*); scarlet (*st*); eosinoid (*es*); and white (*w*). A standard wild type strain derived from a collection at Pasadena, California, served as the control. Each stock was cultured en masse on the usual corn meal, yeast and agar medium at a room temperature of 22–23°C. The flies were raised in quarter pint milk bottles and care was taken to prevent overcrowding by allowing the parental females to oviposit for a maximum of 24 hours. The adult flies were collected at intervals of 0–2 hours after emergence in order to limit the food intake to a minimum. Immediately after collection, the adults were frozen by immersion in liquid air. The flies were kept in the frozen state for at least 30 minutes after which they were transferred to a vacuum desiccator and dried

in vacuo over Al_2O_3. It was found that desiccation for 24 hours was sufficient to dry the flies to a constant dry weight. After complete drying, the flies were pulverized with an agate mortar and pestle and then stored *in vacuo* over Al_2O_3 until used.

In making the tryptophane determinations two methods were used. The initial analyzes were made according to the method of ECKERT (1943). Subsequent tests to determine the specificity of this method showed that it is relatively non-specific. Other compounds, especially those involved in tryptophane metabolism, such as indole and kynurenin, react in a manner similar to tryptophane (CASPARI and GREEN, unpublished) and their presence may lead to excessively high results. While this non-specificity is disadvantageous, it will be pointed out that certain results obtained with this method aid in the clarification of the action of certain of the mutant genes studied. The second method used was the modification of the May-Rose p-dimethylaminobenzaldehyde method developed by HORN and JONES (1945). This method has been shown to be most specific in its reaction with tryptophane. Results obtained by performing duplicate analyses with this method and a bioassay method based on the growth of a tryptophane-requiring strain of *Escherichia coli* were identical (CASPARI and GREEN, unpublished).

Non-protein tryptophane was determined for each mutant by first precipitating the fly proteins with 0.3M trichloracetic acid followed by analysis of the filtrates for tryptophane content.

Total tryptophane was determined after first hydrolyzing the fly proteins. The first attempts at determining the total tryptophane depended upon the alkaline hydrolysis of the proteins. This method, in which the dried flies were autoclaved with 5N NaOH, gave erratic results and was discarded. The procedure finally adopted was a slight modification of the method of GREENHUT, SCHWEIGERT and ELVEHJEM (1946). This method is based on the enzymatic hydrolysis of the proteins. The enzymes used here were a combination of the proteolytic enzymes of dried pancreas (pancreatin) and of dried small intestinal mucosa (duodenin). To fly samples of approximately 50 mgm were added 10 mgm of pancreatin and 5 mgm of duodenin as a solution in phosphate buffer of Ph 8.2. The samples were layered with toluene to prevent bacterial growth and were then shaken in an incubator at 37°C. After 20-24 hours of incubation, the added enzymes were precipitated with trichloracetic acid and the tryptophane content of the filtrates determined by the method of HORN and JONES. In agreement with the report of GREENHUT *et al.*, shaking was necessary for complete hydrolysis. Shaking for less than 18 hours resulted in low tryptophane results, while no increase was found after shaking for longer than 24 hours.

In addition to the tryptophane content, determinations of the non-protein α amino nitrogen and total α amino nitrogen were made on the appropriate filtrates using the method of POPE and STEVENS (1939).

All results presented represent the means of three or more duplicate analyses. Calculations have been made as milligrams of tryptophane or α amino

nitrogen per gram of dried flies. Protein tryptophane and protein α amino nitrogen have been calculated as the difference between the total and non-protein values.

NON-PROTEIN TRYPTOPHANE COMPOSITION

In table 1 are presented the results of non-protein tryptophane determinations by the two methods used as well as the non-protein α amino nitrogen in wild type and mutant *D. melanogaster*. It may be observed that the results obtained for the various mutants by the method of HORN and JONES are essentially in agreement with one another and with wild type, with the exception of *v* and of *su*^{2}*-s, v*. In these two mutants there appears to be a significant excess of non-protein tryptophane. The six-fold increase in the non-protein tryptophane content of *v* in comparison with wild type flies appears to be an increase in this amino acid alone. Examination of the results obtained for α amino nitrogen indicate no general increase in the non-protein amino acids as compared with wild type. A similar increase in the non-protein tryptophane is observed in the case of *su*^{2}*-s, v*, but in this case the increase is not as marked as in *v*.

TABLE 1

Non-protein tryptophane composition of D. melanogaster.
(All results expressed as mgm/gm dried flies.)

GENOTYPE	HORN AND JONES METHOD	ECKERT METHOD	NON-PROTEIN α AMINO NITROGEN
wild type	0.215	0.329	4.82
v	1.389	1.151	4.93
su^2*-s, v*	0.512	0.539	5.13
bw	0.236	0.574	5.36
cn	0.235	1.253	5.39
st	0.250	0.565	5.13
w	0.171	0.306	4.27

The results of non-protein tryptophane determinations as made by the method of ECKERT are essentially in agreement with those found by the method of HORN and JONES. One exception is evident. It will be noted that the results obtained for *cn* by the two methods are in sharp disagreement. As pointed out previously, the ECKERT method is relatively non-specific for tryptophane and such compounds as indole and kynurenin when present could lead to excessively high results. The discrepancy between the values obtained by the two methods undoubtedly results from the presence of kynurenin in the *cn* flies. Kynurenin has been isolated from *cn* pupae (KIKKAWA 1941). Moreover, trichloracetic acid filtrates of *cn* adults when tested for the presence of kynurenin by the OTANI-NISHINO reaction gave a strongly positive test, whereas similar tests performed on wild type and mutants other than *cn* were negative. It will be noted that in the case of all determinations except that for *v*, the results obtained by the ECKERT method were somewhat higher than

those obtained by that of HORN and JONES. It is suspected that in all these cases the higher results may be related to the presence of small amounts of kynurenin in the adult flies.

Non-protein tryptophane results for the wild type and mutants of *D. virilis* are listed in table 2. As in *D. melanogaster*, the results obtained by the HORN and JONES method indicate a significant excess of non-protein tryptophane in *v* as compared with the other mutants. In addition there is a strong suggestion that the non-protein tryptophane content of *w* of *D. virilis* is also significantly greater. This finding differs from the results obtained for the *w* mutant of *D. melanogaster*, where the non-protein tryptophane content was slightly lower than in wild type. It has been suggested that the *w* mutants in the two species are homologous. The finding of this difference may be an indication of the non-homology of the two mutants. However, additional information must be obtained before a conclusive answer can be given to this problem. Similar to the findings for *D. melanogaster*, the results obtained in *D. virilis* using the

TABLE 2

Non-protein tryptophane composition of D. virilis.
(All results expressed as mgm/gm dried flies.)

GENOTYPE	HORN AND JONES METHOD	ECKERT METHOD	NON-PROTEIN α AMINO NITROGEN
wild type	0.201	0.303	4.36
v	0.892	0.842	5.28
es	0.224	0.343	5.57
st	0.285	1.116	4.89
cn	0.223	0.432	4.98
w	0.343	0.598	5.63

method of ECKERT are essentially in agreement with the results obtained by the HORN and JONES method, except for the mutant *st*. This discrepancy can be accounted for by the presence of kynurenin in *st* flies as indicated by a strongly positive OTANI-NISHINO test. These findings support the conclusion of STURTEVANT and NOVITSKI (1941) that *cn* of *D. melanogaster* and *st* of *D. virilis* represent homologous mutations.

PROTEIN TRYPTOPHANE COMPOSITION

Table 3 lists the results obtained for the total tryptophane of wild type and mutant adult flies of *D. melanogaster*. Superficial examination of these values indicates that there is a wide variation in the total tryptophane of *D. melanogaster*. Evaluation of these results will show that the differences found reflect variations in the non-protein tryptophane and in the total protein content of the flies. However, no significant difference in the protein tryptophane composition among the various mutants was found.

The protein tryptophane in the various mutants has been calculated as the difference between the mean total tryptophane and the mean non-protein tryptophane as obtained by the HORN and JONES method (table 3). From the values obtained for the protein tryptophane, the protein tryptophane α amino

nitrogen has been calculated on the basis of tryptophane containing 6.86 percent α amino nitrogen (table 3).

Similarly the protein α amino nitrogen has been calculated as the difference between the mean total α amino nitrogen and the mean non-protein α amino nitrogen (table 3). The protein α amino nitrogen may serve as a measure of the protein content of the flies. On this basis, it would appear that the protein content is somewhat variable. However, the only significant differences are those of approximately ten percent between wild type and *bw* and *v*. Since it happens that the flies of these three genotypes were not raised concurrently, further tests would be necessary to show whether these differences are intrinsic or the result of variations in the culture media.

The percentage tryptophane composition of the fly proteins has been calculated as 100× the ratio of protein tryptophane α amino nitrogen to protein

TABLE 3

Total and protein tryptophane composition of D. melanogaster.
(All results expressed as mgm/gm dried flies except as noted in final column.)

GENOTYPE	TOTAL TRYPTOPHANE	PROTEIN TRYPTOPHANE	TOTAL α AMINO NITROGEN	PROTEIN α AMINO NITROGEN	PROTEIN TRYPTOPHANE α AMINO NITROGEN	PERCENT PROTEIN TRYPTOPHANE COMPOSITION
wild type	5.63	5.42	48.16	43.34	0.37	0.85
v	7.33	5.94	54.02	49.09	0.41	0.83
su^{s}-*s*, *v*	6.32	5.81	51.02	46.89	0.40	0.85
bw	6.23	5.99	55.15	49.79	0.41	0.82
cn	6.05	5.82	53.71	48.32	0.40	0.83
st	6.03	5.78	52.35	47.22	0.40	0.84
w	5.85	5.68	50.31	46.04	0.39	0.85

α amino nitrogen. These results may be found in the last column in table 3. It may be seen that the protein tryptophane composition among the various mutants studied varied from 0.82–0.85 percent. In view of the errors inherent in the analytical procedures used, such a variation is not unexpected and the differences, where they occur, cannot be considered as significant. Therefore it can be concluded that in *D. melanogaster* no significant difference in the protein tryptophane composition in the various mutants studied occurs. Where there are differences, they may be resolved as being the result either of increased non-protein tryptophane, as in the case of *v*, or of an overall increase in the fly proteins.

In table 4, determinations of the total tryptophane content of the mutants in *D. virilis* have been presented. Calculations of the protein tryptophane and the percentage protein tryptophane composition in these mutants have been made exactly as they were made for the mutants of *D. melanogaster*. In the case of *D. virilis*, the protein tryptophane composition varied between 0.95–0.98 percent, indicating that here too there is no significant difference among the mutants studied.

TABLE 4

Total and protein tryptophane composition of D. virilis.
(All results expressed as mgm/gm dried flies except as noted in final column.)

GENOTYPE	TOTAL TRYPTOPHANE	PROTEIN TRYPTOPHANE	TOTAL α AMINO NITROGEN	PROTEIN α AMINO NITROGEN	PROTEIN TRYPTOPHANE α AMINO NITROGEN	PERCENT PROTEIN TRYPTOPHANE COMPOSITION
wild type	6.24	6.04	47.47	43.11	0.41	0.96
v	7.96	7.07	55.87	49.59	0.48	0.96
es	6.80	6.58	53.08	47.51	0.45	0.95
st	7.13	6.85	52.93	48.04	0.47	0.98
cn	7.06	6.84	54.19	49.21	0.47	0.95
w	7.54	7.20	56.84	51.21	0.49	0.96

It may be pointed out that while within each species the protein tryptophane composition is constant, there does appear to be a real difference between *D. melanogaster* and *D. virilis* with respect to protein tryptophane.

DISCUSSION

The results presented here support the conclusion that the eye color mutants of *D. melanogaster* and *D. virilis* do not influence the protein tryptophane composition of the flies. This observation is at variance with the findings reported for *a* and a^+ in Ephestia by CASPARI (1946). It has been suggested that the *v* mutation of Drosophila and the *a* mutation of Ephestia are homologous; it seems justifiable to conclude here that in so far as their effects on the protein tryptophane composition are concerned, the two mutations are not homologous.

Moreover it is apparent that in the case of the *v* mutants of Drosophila, the failure to make brown pigment is not correlated with excessive storage of tryptophane in the proteins. The findings reported here point to a marked accumulation of non-protein tryptophane in the *v* flies, indicating that while there is sufficient tryptophane available during development, *v* flies are completely incapable of carrying out the oxidation of tryptophane to kynurenin. Further support for this conclusion may be drawn from the observation that when the su^2-*s* mutation is introduced into *v* flies, the biosynthesis of brown pigment is correlated directly with the utilization of tryptophane. Thus in su^2-*s*, *v* flies, in which the eyes approach wild type pigmentation, the non-protein tryptophane is reduced to less than 50 percent of the amount found in *v* flies. Since it has been reported that kynurenin is formed in su^2-*s*, *v* flies, though on a reduced scale in comparison with wild type (BEADLE and EPHRUSSI 1936), it follows that the relative drop in non-protein tryptophane in su^2-*s*, *v* as compared with *v* is the direct result of oxidation of tryptophane to kynurenin. The accumulation of tryptophane in *v* flies appears to be comparable to the findings of TATUM, BONNER and BEADLE (1944) in a tryptophaneless mutant of Neuro-

spora. Here it was observed that the mutant strain, which cannot convert anthranilic acid to indole, accumulates anthranilic acid.

Similar results have been reported here with the mutants *cn* of *D. melanogaster* and *st* of *D. virilis*. In these mutants the conversion of tryptophane to kynurenin is carried out, but further step(s) in the biosynthesis of brown pigment fail at the kynurenin level such that kynurenin accumulates in these two mutants.

The data presented here do not warrant any clear-cut conclusions on the mechanism of action of the genes *st* of *D. melanogaster* and *cn* of *D. virilis* nor of the *w* mutations in both species. If the amounts of non-protein tryptophane present in these mutants represent a measure of kynurenin synthesis, then it appears that the failure in brown pigment synthesis occurs in some stage after kynurenin formation. Comparison of the results obtained for non-protein tryptophane by the two methods used indicates that kynurenin is also metabolized. However the data do not suggest at what point between the metabolism of kynurenin and the formation of brown pigment the failure occurs. While the homology between the *st* mutant of *D. melanogaster* and the *cn* mutant of *D. virilis* seems to be supported by the data presented here, there is some question with respect to the homology between the *w* mutations of the two species. It may be noted that the non-protein findings for *w* of *D. melanogaster* indicate the amount of tryptophane present to be the same as or less than that found in wild type flies. Conversely, non-protein tryptophane of *w* in *D. virilis* appears to be significantly greater than that found for wild type. There is the possibility that these results indicate different modes of gene action for the *w* mutations of the two species.

From the results of investigations in Neurospora, it has been suggested that each gene controls a specific metabolic step (HOROWITZ *et al.* 1945). The findings for the *v* and *cn* genes of *D. melanogaster* and the *v* and *st* genes of *D. virilis* fit into such a scheme of gene action. It has been argued that if genes control the production of enzymes, a block in a particular reaction chain should be manifested in the accumulation of the substrate immediately prior to the point of blockage (BONNER 1946). Such accumulations are to be noted here in the case of the *v* mutants and *cn* of *D. melanogaster* and *st* of *D. virilis*. These results are then in agreement with the point of view that such accumulations of substrates constitute evidence for the failure in the elaboration of specific enzymes in the presence of specific mutant genes. On the other hand it might be argued that the failure of the metabolic step is the result of the elaboration of a specific enzyme inhibitor. Unpublished experiments with the *v* mutation of *D. melanogaster* however appear to support the former idea, although more information is necessary before a definite conclusion can be drawn.

SUMMARY

Analyses of the protein and non-protein tryptophane content of adults with wild type and mutant eye color in *D. melanogaster* and *D. virilis* have been presented.

No difference was found in the protein tryptophane composition between the various eye color mutants and wild type within each species. However, a difference in the protein tryptophane content between *D. melanogaster* and *D. virilis* was found.

Non-protein tryptophane was found to accumulate in the *v* mutants of both species. Similarly, kynurenin was found to accumulate in the *cn* mutant of *D. melanogaster* and the *st* mutant of *D. virilis*.

It is concluded that the accumulation of tryptophane and kynurenin in the specified mutants supports the hypothesis that each gene mutation fails to elaborate a specific enzyme necessary for the metabolism of the accumulated compounds.

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MATERNAL EFFECT INVOLVED IN THE INHERITANCE OF ABNORMAL GROWTHS IN THE HEAD REGION OF DROSOPHILA MELANOGASTER

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AN abnormality expressed as irregular growths in the head region of *Drosophila melanogaster* is under investigation in the genetics laboratory at the UNIVERSITY of UTAH. The experimental evidence indicates that a maternal effect controlled by a sex-linked gene is involved in the expression of the abnormality. In addition to the maternal effect an autosomal gene is required for the expression of the character.

MATERIALS AND METHODS

The study involves a stock of flies, carrying an inherited abnormality with a varied expression, characterized by growths in the surface of the head. Some examples of the growths are shown in semidiagrammatic drawings in figure 1. The growths are irregular in shape and size and occur in different regions. They are distributed mainly in the areas normally occupied by the eye, face, and antenna. A size gradation has been observed from very small irregularities involving only a few facets of the eye or a small area elsewhere on the head to massive abnormalities, in which both eyes are involved and most of the head is distorted by amorphous growths. A detailed description of the different expressions is being prepared by W. W. NEWBY. The trait first appeared in the spring of 1945 at the UNIVERSITY OF TEXAS in a sample from a wild population which had been collected at Acahuizotla, Mexico in 1941. Flies showing the abnormality were received at the UNIVERSITY OF UTAH in the fall of 1946 through the courtesy of DR. WILSON STONE. The abnormal flies have been inbred during the past two years at the UNIVERSITY OF UTAH. A slight increase has been observed in the penetrance and expressivity following selection. At present the inbred stock shows an average penetrance of about 76 percent at 22°C.

The name *tumorous head* suggested itself to the writers early in the study as a suitable descriptive term to identify the character. This name was used in preliminary reports (GARDNER, in press, WOOLF, in press) and is used here to designate the character and the stock which carries it. The word tumor is used in a broad sense to mean enlargement or growth and there is no intention to imply a relationship with any other character described in Drosophila or any other animal group for which the same name has been used. Other characters in Drosophila have been identified with the same word (STARK 1919; BRIDGES and BREHME 1944) but there seems to be no relationship between these and the trait discussed here. The symbol *tu-h* is used to symbolize the tumorous head stock. The symbols *tu-1* and *tu-3* are designated tentatively to symbolize the sex-linked gene which controls the maternal effect and the third

chromosome gene respectively. In diagrams of crosses where chromosomes are identified, arabic numbers are used to symbolize the chromosomes of the *tu-h* stock. In cases where the parents of a cross are symbolized, the female parent is always written first. The flies were raised in half-pint milk bottles on the standard cornmeal, agar, mollasses media at 22°C.

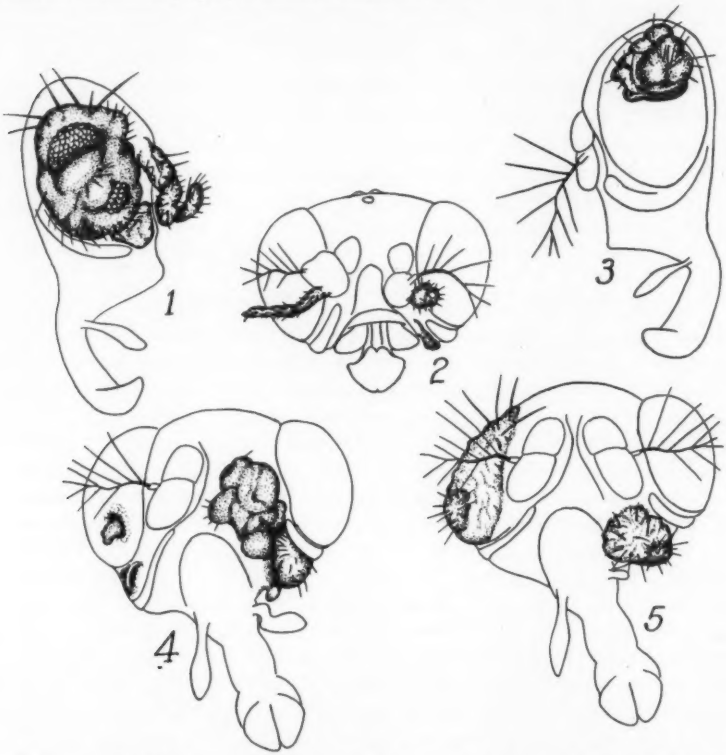

FIGURE 1.—Semidiagrammatic drawings of head growths in *tu-h* stock. The stippled areas represent the abnormalities. 1. Side view of head showing massive amorphous abnormality involving most of right eye, lower face and region of the antenna. 2. Face view showing three small growths. The one originating in the base of the right antenna is slightly leg-like. 3. Side view showing a single growth of medium size in upper part of left eye. 4. Face view showing three growths of different sizes. 5. Face view showing two growths. One covers the area normally occupied by the entire right eye.

EXPERIMENTAL RESULTS

Genetic Mechanism Involved

Results of reciprocal crosses involving *tu-h* and stocks with marked chromosomes indicate that two major loci are involved in the production of the abnormality. A sex-linked gene (*tu-1*) is responsible for the maternal effect and a third chromosome semidominant gene (*tu-3*) is also required for the expression

of the abnormality. These facts were demonstrated by the use of flies prepared from the *tu-h* stock by appropriate crosses which contain suitable dominant markers for the identification of the different chromosomes. The markers provided a means of eliminating the chromosomes from the *tu-h* stock one by one and thus determining which were necessary for the production of the abnormality. The essential crosses are reconstructed and the data are summarized in table 1.

Crosses 1–4 (table 1) were matings between flies prepared by crossing $\frac{ClB}{+}\frac{H}{Sb}$ females with *tu-h* males$\left(\frac{tu\text{-}1}{-}\frac{tu\text{-}3}{tu\text{-}3}\right)$. The F_1 females $\left(\frac{ClB}{tu\text{-}1}\frac{H}{tu\text{-}3}\text{ and }\frac{ClB}{tu\text{-}1}\frac{Sb}{tu\text{-}3}\right)$ were backcrossed individually to *tuh* males.

From the resulting progenies flies were selected which were known to be homozygous for *tu-1* and were also known to carry one or two *tu-3* chromosomes as indicated by the markers $\left(\frac{tu\text{-}1}{tu\text{-}1}\frac{H}{tu\text{-}3}, \frac{tu\text{-}1}{tu\text{-}1}\frac{Sb}{tu\text{-}3}, \frac{tu\text{-}1}{tu\text{-}1}\frac{tu\text{-}3}{tu\text{-}3}\text{ etc.}\right)$

These were mated together as shown diagrammatically in crosses 1–3. From the progeny of cross 3 flies expressing Hairless and Stubble were selected and mated as shown in cross 4.

The results of these four crosses demonstrate the fact that *tu-3* is located in the third chromosome. From cross 1 an average of 74 percent of abnormal flies was obtained. This is approximately the same average percentage of abnormal flies as that produced by the inbred *tu-h* stock. Both major factors were present and the cross was essentially parallel with those involving flies from the inbred *tu-h* stock. Cross 2 was similar but carried only three *tu-h* third chromosomes compared with four in cross 1 and produced an average of 46 percent abnormality compared with 74 percent for cross 1. Cross 3 carried only two *tu-h* third chromosomes and an average of 32 percent of abnormal flies was obtained. Cross 4 had no *tu-h* third chromosomes and produced no abnormal flies. These results show that in the presence of the sex chromosomes from the *tu-h* stock the percentage of abnormal flies was roughly proportional to the number of *tu-h* third chromosomes present.

Further evidence for the location of *tu-3* in the third chromosome and for its behavior as a semidominant was obtained by classifying the progeny from crosses 2 and 3. Cross 2 carried only one marker (*Sb*) and three *tu-h* third chromosomes. Half of the progeny would be expected to carry only one *tu-3* and these would express the Stubble character. Half would be homozygous for *tu-3*. Only 21 percent of the Stubble flies were abnormal compared with 80 percent for those not Stubble. Two markers (Hairless and Stubble) were present in cross 3 and a larger number of progeny was obtained. The results showed that 18 percent of the flies expressing Hairless and 15 percent of those expressing Stubble were abnormal while 65 percent of those expressing neither

marker, and therefore homozygous for *tu-3*, were abnormal. No abnormal flies were found to express both markers (Hairless and Stubble). These flies would not have any *tu-3* genes and the results indicate that the expression does not occur in the absence of this gene. The results of crosses 1–4 prove that *tu-3* is semidominant and is located in the third chromosome. The results show further that *tu-3* is homozygous viable under the conditions of the experiment at 22°C.

TABLE 1

Crosses designed to demonstrate the location and action of genes involved in the expression of abnormal growths and the results of these crosses.

	Cross	Bar or Non-bar	Abn ♀	+♀	% Abn ♀	Abn ♂	+♂	% Abn ♂	Avg. % Abn
1.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$		645	126	84.0	516	291	64.0	74.0
2.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{Sb}$		81	62	57.0	70	112	38.0	46.0
3.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{H} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{Sb}$		275	378	42.0	161	530	23.0	32.0
4.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{Sb}{H} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{Sb}{H}$		0	149	0	0	147	0	0
5.	$\frac{ClB}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$	Bar Non-bar	19 29	656 641	2.8 4.3	10	666	1.5	2.9
6.	$\frac{ClB}{tu\text{-}1}\ \frac{Sb}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{H}{tu\text{-}3}$	Bar Non-bar	6 10	407 338	1.5 2.9	6	321	1.8	2.0
7.	$\frac{ClB}{+}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$	Bar Non-bar	1 0	97 85	0 0	0	84	0	.4
8.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{+}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$		131	21	86.0	95	37	72.0	80.0
9.	Lausanne $\times \frac{+}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}$		1	859	.12	1	835	.12	.12
10.	Lausanne $\times \frac{tu\text{-}1}{\rightarrow}\ \frac{Sb}{H}$		0	814	0	0	881	0	0
11.	$\frac{+}{+}\ \frac{H}{Sb} \times \frac{+}{\rightarrow}\ \frac{H}{Sb}$		0	145	0	0	123	0	0

A maternal effect was indicated by the differential results of preliminary reciprocal outcrosses and has been demonstrated by further studies (see table 3). Proof that a sex-linked gene is responsible for the maternal effect was obtained by making use of a stock carrying *tu-3* and a marker in the first chromosome. Flies used for crosses 5 and 6 (table 1) were prepared as described above

for crosses 1–3 but females carrying *ClB* instead of those without the marker were selected. Parents in cross 5 were both homozygous for *tu-3*. The female carried one sex chromosome from the *tu-h* stock and a *ClB* chromosome containing a marker, Bar eye, (*B*), a crossover suppressor (*C*) and a lethal (*l*). The results showed an average of 2.9 percent of abnormal flies. No Bar eyed males were obtained in the presence of the pseudodominant lethal (*l*). Cross 6 was comparable but carried only two *tu-3* genes instead of four and an average of 2 percent of abnormal flies was produced.

Results of crosses 5 and 6 compared with those of crosses 1 and 3 show that a sex-linked gene is involved. Crosses 1 and 5 differ only in the number of *tu-1* genes present in the female parent. The difference between two and one *tu-1* genes results in a reduction from 74 percent to 2.9 percent of abnormal flies. Likewise, crosses 3 and 6 are comparable. The difference between two and one *tu-1* genes in this combination results in a reduction from 46 percent to 2 percent of abnormal flies. Both Bar eyed and non-Bar eyed females expressed the abnormality in about equal proportion.

Parent flies used in cross 7 were prepared by crossing females carrying dominant markers with *tu-h* males as follows:

$$\frac{ClB}{+}\ \frac{H}{Sb} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{tu\text{-}3}{tu\text{-}3}.$$

F_1 females showing Stubble were then crossed with F_1 males showing Hairless·

$$\frac{ClB}{tu\text{-}1}\ \frac{Sb}{tu\text{-}3} \times \frac{+}{\rightarrow}\ \frac{H}{tu\text{-}3}.$$

The parent flies used in cross 7 were selected from the F_2 progeny. It will be observed (table 1) that this cross is like cross 1 except for the complete absence of *tu-1* in the female parent. One abnormal fly was obtained in a total of 267.

Results of crosses 5, 6, and 7 compared with those of crosses 1, 2, and 3 demonstrate the fact that the maternal effect is controlled by a sex-linked gene (*tu-1*). Comparatively little effect can be attributed to *tu-1* in heterozygous condition. It is therefore recessive or nearly so. Comparison between the results of crosses 5 and 7, however, indicate that *tu-1* may not be completely recessive in cross 5. The parents in crosses 5 and 7 differed only in one respect. The female in cross 5 was heterozygous for *tu-1* and the female in cross 7 carried no *tu-1* genes. The difference between 2.9 percent and .4 percent suggests that *tu-1* may have a slight maternal effect in heterozygous condition. The absence of *tu-1* in the male makes no difference as shown by the results of cross 8. This cross differed from cross 1 only in one respect. The male from cross 8 (obtained from the progeny of cross 7) carried no *tu-1* gene. The percentage of abnormal flies (80 percent) was slightly above that from cross 1 (74 percent) and that from the inbred *tu-h* stock (76 percent) but the difference was not considered significant.

A small percentage (less than 1 percent) of abnormal flies was obtained

from cross 7 which carried no *tu-1* genes in the female parent. A few abnormal flies were also obtained from outcrosses between *tu-h* males and females from laboratory stocks (see table 3). No maternal effect would be expected in these crosses and the small percentage could not be explained on the basis of the interaction described above. It was presumed that either *tu-1* or *tu-3* must have a slight independent action when introduced by the male. Crosses 9, 10, and 11 were designed to test this possibility and indicate the source of this small expression. The Lausanne stock which had been shown to behave like most other laboratory stocks in crosses with *tu-h* was chosen for the experiment. Lausanne females were crossed with males homozygous for *tu-3* but not carrying a *tu-1* gene as shown in cross 9. The male parent in this cross was like the one used in cross 8 and was obtained from the same source. Lausanne females were also crossed with males carrying *tu-1* but not *tu-3* as shown in cross 10. The male in this cross was like the one used in cross 4 and was prepared in a similar way.

TABLE 2

Crosses designed to test the second chromosome and results of these crosses.

	CROSS	Abn ♀	+♀	Abn ♂	+♂	% Abn.
1.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{2}{2}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{2}{2}\ \frac{tu\text{-}3}{tu\text{-}3}$	385	65	298	165	75
2.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{2}{Cy}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{2}{Cy}\ \frac{tu\text{-}3}{tu\text{-}3}$	208	57	172	113	69
3.	$\frac{tu\text{-}1}{tu\text{-}1}\ \frac{Cy}{Pm}\ \frac{tu\text{-}3}{tu\text{-}3} \times \frac{tu\text{-}1}{\rightarrow}\ \frac{Cy}{Pm}\ \frac{tu\text{-}3}{tu\text{-}3}$	251	85	204	152	66

From a total of 1,696 flies resulting from cross 9, two were abnormal. The evidence is meager but does indicate that the small percentage of abnormality from crosses involving *tu-h* males and females not carrying *tu-1* was produced by the independent action of *tu-3*. The results of cross 10 show that no abnormal flies were produced in a total of 1,695. This indicates that *tu-1* has no independent action when introduced by the male. In cross 11 both *tu-1* and *tu-3* were absent and no abnormality was produced, as would be expected.

The above crosses prove that two major genes are involved and that they are located in the first and third chromosomes. Appropriate crosses were made to determine whether or not modifiers are located in chromosomes other than the first and third in the inbred *tu-h* stock. Crosses designed to test the second chromosome are given in table 2 along with the results obtained. Flies used in all three crosses were homozygous for both major genes and differed only in the number of *tu-h* second chromosomes present. Parents in cross 1 each carry two *tu-h* second chromosomes. The percentage of abnormal flies was comparable with that of the inbred *tu-h* stock as would be expected. Each parent in cross 2 carried only one *tu-h* second chromosome and 69 percent of the progeny were

abnormal. All second *tu-h* chromosomes were removed from cross 3 and 66 percent of abnormal flies was obtained. These results suggest that a second chromosome modifier is present in the *tu-h* stock and that it has a slight enhancing effect. Wide variation is observed in the results of single crosses even in the inbred *tu-h* stock and therefore the significance of this difference is not clear. Further study is necessary before a conclusion can be drawn. The average penetrance of the *tu-h* stock has increased about 10 percent during the two year period of inbreeding, suggesting that modifiers with a slight enhancing effect have been accumulated.

In all of the results cited in tables 1 and 2 it will be observed that a higher percentage of abnormality occurs among the females than among the males from the same cross. This is also true of the inbred stock and was observed in outcrosses as well. The degree of expression or extent of the abnormality in individual flies is also greater among the females than among the males. The possibility that *tu-1* has a direct action in addition to the maternal effect which is exerted in greater intensity in homozygous females than in simplex males has been considered as a possible explanation. Evidence against this explanation comes from the fact that the usual pattern expected from a sex-linked gene with direct action has not been observed in the results of out-crosses. Males and females are produced in a regular proportion regardless of the segregation of the sex chromosomes. This differential suggests that the expression is associated in some way with sex.

Maternal Effect Shown in Results of Reciprocal Outcrosses

Results of reciprocal outcrosses showed that a maternal effect is involved in the production of the head growths. A considerably greater percentage of abnormality occurred regularly in the F_1 of a cross between a *tu-h* female and a male from another stock than in the F_1 from the reciprocal cross. The results of reciprocal outcrosses with laboratory stocks are given in table 3. Only the total flies and average percentage of abnormality in the progeny from each cross are given, with accuracy to the nearest whole number. Four wild stocks and eleven other laboratory stocks carrying mutants in different chromosomes were included in the study. The F_1 results summarized in table 3 show that an average of about 30 percent of the F_1 progeny from the outcrosses involving females from the *tu-h* stock was abnormal. The females from the *tu-h* stock were expected to be homozygous for *tu-1*. This condition would be expected to produce the maternal effect. The F_1 progeny would be heterozygous for *tu-3* making a combination which might be expected on the basis of results shown in table 1 to produce about 30 percent of abnormal flies. These outcrosses would be essentially similar to cross 3 (table 1). In the outcrosses, however, two *tu-3* genes would enter through the female while in cross 3 one *tu-3* gene was introduced by each parent. A rather wide range, from 14 percent to 52 percent of abnormal flies, was observed, from single crosses, but a wide range of expression occurred also in the inbred *tu-h* stock. With one exception (Oregon) the reciprocal crosses involving males from the *tu-h* stock resulted in progeny less than 1 percent of which were abnormal. This indicates clearly that a

maternal effect is involved. Abnormal males and abnormal females were obtained in the results of both reciprocal crosses in all cases in which the ab-

TABLE 3

Average percentage of abnormality from reciprocal outcrosses between tu-h and laboratory stocks.

LABORATORY STOCK	F_1				F_2			
	tu-h× LAB. STOCK		RECIPROCAL CROSS		*tu-h*× LAB. STOCK		RECIPROCAL CROSS	
	*n	**%	n	%	n	%	n	%
Oregon R.	817	38	1,396	42	1,621	20	1,728	26
Canton	750	25	1,542	0	540	0	329	0
Florida	107	52	220	1	96	4	287	1
Wild (Turtox)	807	19	947	1	706	1	1,275	1
w	197	19	583	1	293	2	223	1
al dp d b c px sp	96	33	78	0	1,304	9	1,122	10
ss^a	167	49	598	0	720	0	497	0
e	255	20	96	0	197	1	351	0
Bd^a	157	20	53	0	328	2	167	5
In(3R)C l(3)e	216	19	42	0	236	4	341	3
Muller 1^{13}	361	37	289	0	531	1	537	1
Cy/Pm; H/Sb	90	39	88	1	1,580	1	320	1
D/G1	170	14	132	0	956	4	106	1
M(3) 124/In(3R)C e l(3)e	101	23	35	0	695	0	423	1
ey^D*/or*	64	37	27	0	173	0	cross failed	

* n = total flies.

** % = percentage of abnormality with accuracy to nearest whole number. Less than .5% is recorded as 0.

normality was present but abnormal females were present in a higher proportion than abnormal males.

The F_2 results (table 3) were comparable for reciprocal crosses. Small percentages of abnormal flies not exceeding 5 percent, were observed in the results of all crosses except those involving Oregon and *al dp d b c px sp*. The

high percentages from the Oregon crosses are interpreted further on. About 10 percent of abnormal flies was obtained in the F_2 from both reciprocal crosses involving *al dp d b c px sp* suggesting the presence of a modifier. Since the F_1 results from this cross show the maternal effect and conform to the usual pattern the modifying factor in the *al dp d b c px sp* stock must influence the reaction dependent upon *tu-3* directly.

The comparable F_2 results from reciprocal crosses, showing only slight expression, indicate that little if any maternal effect occurs in the second generation. This would be expected since the maternal effect is dependent upon the genotype of the female parent in the immediate cross. The F_1 combination (*tu-1*/+) is incapable of producing the maternal effect in appreciable degree as indicated by crosses 5 and 6, table 1. The eggs destined to produce the F_2, therefore, would not carry much maternal effect, if any, and the low percentages of abnormality would be expected to result mostly from the independent action of *tu-3*. Some of the F_2 progenies produced as much as 5 percent of abnormal flies which is more than would be expected from the usual independent action of *tu-3*. This suggests the presence of modifiers in the stocks concerned. The F_2 results give further evidence for the maternal effect and the genetic mechanism described above.

The explanation is further borne out by F_3, F_4, F_5 and backcross results. It was observed, for example, that F_3 progeny from original crosses between *tu-h* females and males from laboratory stocks (other than Oregon) produced about 10 percent of abnormal flies while the F_3 from the original reciprocal cross (*tu-h* male × laboratory stock) produced less than 1 percent of abnormal flies. The F_4 from this latter cross was comparable with the F_3 of the former cross. The following explanation will account for this difference. The F_1 progeny from all *tu-h* females showed the maternal effect as described above. The F_1 females were heterozygous for *tu-1* and, therefore, were incapable of producing much maternal effect through their eggs. Some F_2 females received the *tu-1* gene in homozygous condition and produced eggs from which abnormal F_3 flies were produced. On the other hand the F_2 flies from the reciprocal P_1 cross could not be homozygous for *tu-1* and, therefore, would not show an appreciable expression in the F_3. Some F_3 females, however, could be homozygous for *tu-1* and would be expected to produce the substance in their eggs making it possible for the F_4 progeny to express the trait in about the same proportion as the F_3 in the cross above. F_4 and F_5 results from both crosses are comparable and give evidence of the segregation of *tu-1* and *tu-3*.

PRESENCE OF *tu-1* IN OREGON R STOCK

The result of the cross between Oregon R females and *tu-h* males was the only one which differed markedly from the F_1 pattern represented in table 3. In this case the reciprocal crosses gave a comparable percentage of abnormal flies. Therefore, no maternal effect was in evidence when this cross was considered alone. High percentages of abnormality were also recorded for the F_2 of both reciprocal crosses, in marked contrast with the usual F_2 pattern. These deviations suggested that Oregon females are like *tu-h* females in their

ability to influence the expression of the abnormality. This similarity was demonstrated by crossing Oregon flies reciprocally with *tu-h* flies carrying third chromosome markers. The crosses are reconstructed and the results are given in table 4. It will be observed that Oregon females behaved like *tu-h* females and produced a maternal effect in the presence of *tu-3* introduced by the male. When *tu-3* was not present in the male no abnormality was obtained. The reciprocal crosses showed the maternal effect from *tu-h* only in the presence of *tu-3* in the female. These results indicate that Oregon carries a potential maternal effect but lacks the other partner necessary for the expression of the abnormality. Therefore, the inbred Oregon stock does not express the phenotype expressed by *tu-h*. From this evidence it was postulated that Oregon carries *tu-1* or an allele capable of producing a comparable maternal effect but does not carry *tu-3*.

TABLE 4

Crosses designed to demonstrate the maternal effect from Oregon R stock and the results of these crosses.

CROSS	Abn. ♀	+♀	Abn. ♂	+♂	% Abn.
1. Ore × $\frac{tu\text{-}1}{\rightarrow}$ $\frac{tu\text{-}3}{tu\text{-}3}$	17	21	19	22	46
2. Ore × $\frac{tu\text{-}1}{\rightarrow}$ $\frac{H}{Sb}$	0	177	0	187	0
3. $\frac{tu\text{-}1}{tu\text{-}1}$ $\frac{tu\text{-}3}{tu\text{-}3}$ × Ore	63	70	47	72	44
4. $\frac{tu\text{-}1}{tu\text{-}1}$ $\frac{H}{Sb}$ × Ore	0	187	0	191	0

The possibility that the maternal effect in Oregon is controlled by *tu-1* or an allele of *tu-1* was examined further through a series of backcrosses summarized in table 5. It will be observed that the F_1 females from both reciprocal crosses between *tu-h* and Oregon behaved like females from the Oregon and *tu-h* inbred stocks. The differences in percentage of abnormality observed in the different backcross results can be explained on the basis of the number of *tu-3* genes present. Comparable backcrosses involving the Canton stock, which does not produce the maternal effect, are given in table 5 for comparison. Since the F_1 females having received one sex chromosome from Oregon and one from *tu-h* have the same general effect as the females from either parental stock it is concluded that *tu-1* or an allele of *tu-1* is present in Oregon. It should be pointed out here that the expression of the abnormality has never been observed in the Oregon stock. The maternal effect, therefore, has no independent action in producing the tumorous head phenotype but acts only in the presence of *tu-3*. It follows that the small percentage of abnormal flies resulting from crosses between *tu-h* males and females from

laboratory stock (table 3) likely results from the independent action of *tu-3* as described above.

Results in the F_3, F_4, and F_5 from original reciprocal crosses between *tu-h* and Oregon were also comparable. From one series of 26 F_3 and F_4 crosses originating from *tu-h* × Oregon and Oregon × *tu-h*, which were made at random (parents not selected for the abnormality), 20 progenies showed some expression (ranging from 5 percent to 70 percent of abnormal flies). All female parents were expected to produce the maternal effect and the difference in expression was explained on the basis of the segregation of *tu-3*. Similar

TABLE 5

Average percentage of abnormality from backcrosses involving Oregon and tu-h compared with those involving Canton and tu-h.

PARENTS (P_1)		BACKCROSS TO *tu-h*				BACKCROSS TO OTHER STOCK			
		*F_1 × *tu-h*		*tu-h* × F_1		F_1 × P_1		P_1 × F_1	
FEMALE	MALE	**n	***%	n	%	n	%	n	%
tu-h	Oregon	649	42	409	35	120	22	380	19
Oregon	*tu-h*	333	56	167	64	134	19	372	29
tu-h	Canton	212	0	897	36	224	0	262	0
Canton	*tu-h*	605	0	570	50	266	0	231	0

* Female parent is always written first.

** n = total flies.

*** % = percentage of abnormality with accuracy to nearest whole number. Less than 0.5% is recorded as 0.

evidence of segregation was observed in the F_3, F_4, and F_5 results from other series of crosses involving *tu-h* and laboratory stocks other than Oregon. However, in these series of crosses there was a lower percentage of individual progenies expressing the trait and a lower percentage of abnormality was obtained in most progenies. For example, in a series of 28 F_3 and F_4 crosses between *tu-h* and $\frac{M(3)\ 124}{In\ (3R)C\ e\ l(3)e}$ only 8 progenies showed any expression of the trait. Both *tu-1* and *tu-3* were segregating in these latter crosses.

DISCUSSION

The pattern observed in the present study is that of a genetic mechanism involving a sex-linked gene which is recessive, or nearly so, and a third chromosome semidominant gene. It is complicated by the fact that the sex-linked gene has no direct effect on the flies which carry it but acts only through the mother by creating a change in the egg before fertilization. The nature of the change is unknown but it seems likely that a substance of some kind is involved. The substance presumably is accumulated in the egg cytoplasm at an early stage while the egg is still in the ovary. The genotype of the mother, therefore, determines the condition of the egg which will reflect its influence

into the development of the phenotype of the immediate progeny regardless of the sex-linked *tu-h* genes actually present in the fly. In order to produce this substance in appreciable quantity the mother must carry the sex-linked gene in homozygous condition. The only stock other than *tu-h* found so far to be capable of producing the cytoplasmic substance is Oregon R.

The situation is further complicated by the fact that the cytoplasmic substance is ineffective when alone. The abnormality is produced only through the interaction between this substance and a third chromosome, semidominant gene. This autosomal semidominant is homozygous viable at 22°C. Thus the immediate genotype of the fly is responsible for one partner in the interaction and the genotype of the mother, for the other partner. The more extreme expression in the females from all crosses suggests that the sex of the fly has some influence on the expression of the character.

There are some examples in the literature which bear a resemblance to the present situation. TOYAMA (Review by DOBZHANSKY 1941) has described maternal effects in the silkworm which resembles the one discussed here but they are not as complex. Different races of moths produce eggs with different visible characteristics. The eggs of a given female are all alike and always conform to the pattern of the race or genotype to which the female belongs. The characteristics of the egg are thus determined by the genotype of the mother in whose body the eggs develop. The maternal effect observed in the present study also resembles the case of dextral and sinistral snails described by BOYCOTT *et al.* (1930) and STURTEVANT (1923) but again is more complex. The coiling of the shell of the snail is dependent upon a gene which acts through the cytoplasm of the egg. Again in this case the immediate phenotype is controlled not by the individual snail's own genes but by the genes of the mother.

DOBZHANSKY (1935) has described a maternal effect expressed in the hybrids between race A and race B of *Drosophila pseudoobscura*. When a female from race B is crossed with a male from race A the F_1 males are sterile and have small testes. From the reciprocal cross sterile males are also produced but they have testes of normal size. The development of the abnormally small testes was shown to be the result of an interaction between the cytoplasm of the eggs deposited by a race B mother and the chromosomes of race A introduced by the sperm. This example has a greater resemblance to the present case than those cited above but there is one striking difference. Race A and B represent taxonomically distinct populations which are well on the way toward species formation. All hybrid males from either of the reciprocal crosses are sterile. It is not difficult to visualize how incompatibility and abnormality might arise in hybrids of widely separated groups. GOLDSCHMIDT (1934) has observed that cytoplasmic influence in general seems to increase with taxonomic difference. Most cases of cytoplasmic influence (Review by CASPARI 1948) also follow crosses between members of widely separated taxonomic groups. Very few cases have been reported in which differential action of the cytoplasm is found by crossing different mutants of one species. In the present case the two genes involved appear to represent independent mutations which have come

together in the same stock and are being maintained together. In fact, it seems possible that the *tu-h* stock first originated at the UNIVERSITY of TEXAS through a spontaneous mutation which produced the third chromosome semidominant in the sample of the wild Mexican population which already carried *tu-1*.

SUMMARY

The inheritance of an abnormality characterized by irregular growths in the head of *Drosophila melanogaster* has been analyzed. Two major genes are involved. One is a sex-linked recessive or slightly dominant gene with a maternal effect. The other is semidominant, homozygous viable at 22°C, and is located in the third chromosome. The inbred (*tu-h*) stock at present has an average penetrance of 76 percent at 22°C. An average of about 30 percent of abnormal flies is produced in the F_1 when *tu-h* females are crossed with males from laboratory stocks. Less than 1 percent of abnormal flies result from the reciprocal cross. The F_2 results were comparable for reciprocal crosses and only small percentages of abnormality were obtained. The Oregon R stock carries the sex-linked gene which produces the maternal effect but lacks the third chromosome gene and, therefore, does not express the abnormality.

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ON IMMUNOGENETIC RELATIONSHIPS BETWEEN THE ANTIGENIC CHARACTERS SPECIFIC TO TWO SPECIES OF COLUMBIDAE[1]

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PREVIOUS papers from this laboratory have described the isolation and identification in unit-form of the antigenic characters of the red blood cells whereby Pearlneck (*Streptopelia chinensis*) and Senegal (*St. senegalensis*), respectively, differ from Ringdove (*St. risoria*) (IRWIN 1939; IRWIN and CUMLEY 1947). The cellular antigens specific to the respective species are gene determined, but whether only one gene, or several on a single chromosome, are involved in the production of each of these characters is an open question. From the immunological identity or similarity of these antigenic components can be deduced the relationships of the genes producing the characters specific to each of these two species. Such studies form the basis of this paper.

MATERIALS AND METHODS

The details of the production of antisera, the technics of agglutinin-absorption and of the agglutination procedure have been described in previous papers (IRWIN and COLE 1936, 1937). With minor modifications, these are typical of the methods used in the present experiments.

A single antiserum (163S1) to Pearlneck cells has been used in all these tests. This was possible because the serum was lyophilized (dried in a vacuum) early in 1941, providing a source of antiserum which up to the present has shown only a very minute diminution in antibody content. In contrast, several antisera to Senegal cells have been employed from time to time, no one of which has been as satisfactory for the detection of the antigenic characters peculiar to Senegal as the Pearlneck antiserum has been for identifying those peculiar to Pearlneck. As certainly is to be expected, each antiserum to Senegal appeared to have its own peculiar content of antibodies, so that in making comparisons of various antigenic complexes, it was advisable to select different antisera of about the same specificities.

The reactions summarized in the accompanying tables represent a summary of tests which of necessity have been spread over a period of several years. As a consequence, many of the reactions have been done repeatedly and usually with consistent results. There are a few reactions, however, chiefly involving

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the s-2 antigen, which should be repeated. The limitation in this respect has been that for several years no backcross birds carrying only s-2 have been available.

It was not possible at any one time to make all the combinations of a single reagent with each of the various cells with which it was tested. Therefore, at different trials cells were often used from different backcross birds carrying the same antigenic character. These cells, however, had previously been tested by reciprocal absorptions and subsequent agglutinations for identity of the respective specific substances. Except for possible differences in sensitivity of the cells from different individuals, the results from the use of cells carrying the same antigen but from different birds should be equivalent. A definite fractionation of two of the specific substances (d-4 and s-6) has been noted; however, the cells from the birds involved in these fractionations have not been used in the present comparisons.

EXPERIMENTAL

Summary of previous findings

The cellular constituents peculiar to Pearlneck, in contrast to Ring dove, are called d-1, d-2, d-3 · · · d-12, the letter "d" indicating dove (IRWIN 1939). Those particular to Senegal as compared with Ring dove are termed s-1, s-2, s-3, s-6, · · · s-12, the letter "s" denoting substances peculiar to Senegal (IRWIN and CUMLEY 1947). In this paper, unless otherwise stated, all references to the antigenic characters specific to either Pearlneck or Senegal will imply that these are in contrast to Ring dove. The criterion for recognition of each of these antigenic characters in unit-form is that, in matings to Ring dove, a backcross individual carrying such a character has produced progeny either with or without the cellular substance, in approximately equal proportions. While each of these specific components has behaved in inheritance as would be expected if it were effected by a single gene, the same results could have been obtained if each component were produced by the joint action of two or more genes on a single chromosome originally derived from either Pearlneck or Senegal, and crossing over with a chromosome of Ring dove rarely, if ever, took place. For example, if two or more genes on a single chromosome were closely linked, crossing over with a chromosome of Ring dove would be infrequent, depending upon the closeness of the linkage. On the other hand, if the chromosomes carrying genes with species-specific effects were more or less incompatible with the chromosomes of the other species, by virtue of such genes in either species, crossing over might well be inhibited for a part or all of the length of any such chromosomes. In the opinion of the author, each of the antigenic characters peculiar either to Pearlneck or to Senegal is produced by the joint action of two or more genes. Experimental evidence for this opinion, however, is not available except for a single antigenic component in each species, as will be described.

Following the isolation as single entities of the antigenic characters of Pearlneck it was observed that the cells of Senegal contained at least a part of each of the substances peculiar to Pearlneck (IRWIN and COLE 1940). This relation-

TABLE 1

A comparison of the cellular antigens specific to Pearlneck and Senegal, respectively, as contrasted with Ring dove, based on their reactions with reagents prepared from a Pearlneck antiserum.

CELLS TESTED	ANTI-PEARLNECK SERUM ABSORBED WITH THE CELLS OF																						
	RING DOVE ONLY	RING DOVE CELLS PLUS THOSE OF																					
		Sen.	d-1	d-2	d-3	d-4	d-5	d-6	d-7	d-9	d-11	d-12	s-1	s-2	s-3	s-6	s-7	s-8	s-9	s-10	s-11	s-12	s-2, s-12	LINE
Ring dove	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pearlneck	8, 9	8, 9	8	8	8	8	9	8	9	9	8	9	8, 9	8, 9	9	7, 9	8, 9	7, 9	9	9	9	9	+	2
Senegal	7, 8	0											6	7		7	7	6						3
d- 1	6, 7	0, ?	0										+	0, ±	+	+	+	+	+	+	+	+	0, +	4
d- 2	4, 6	+		0									+	+	+	+	+	+	+	+	+	+	+	5
d- 3	3, 4	0			0				0			±	+	±	+	+	+	+	+	+	0	+		6
d- 4	3, 4	0				0				0			+	+	+	0	+	+	+	±	+	+		7
d- 5	5, 7	0					0						0, ?	+	+	+	+	+	+	+	+	+		8
d- 6	2, 3	+						0				±	±	±	±	+	+	+	±	±	+	±		9
d- 7	3, 4	0, ?			0, ±				0				+	+	+	+	+	+	+	±	?	+		10
d- 9	3, 4	0				0				0			+	+	+	0, ?	+	+	±	±	+	+		11
d-11	8, 9	7, 9									0		C	+	+	+	C	+	+	C	C	C		12
d-12	2, 3	?	±	±	±	+	±	?	±	±	±	0	±	±	±	±	+	±	0	±	+	+		13
s- 1	4, 6	0	+	+	+	+	0, ±	+	+	+	+	+	0	+	+	+	+	+	+		+	+	+	14
s- 2	6	0	②	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+		+	+	0	15
s- 3	0, ?	0													0									16
s- 6	3, 4	0	±	±	+	0	+	+	+	0	+	+	±	±	±	0	±	±	±		±	±		17
s- 7	3, 4	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+		+	+	+	18
s -8	4, 5	0	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	0	+		+	+	+	19
s- 9	2, 3	0	±	±	±	±	±	±	±	±	±	0	±	±	±	±	±	±	0		±	±		20
s-10	0, ?																			0				21
s-11	3, 4	0	+	+	0	+	+	+	0	+	+	+	+	+	±	±	+	+	+		0	+	+	23
s-12	3, 5	0	0	+	±	+	+	+	+	+	+	+	+	±	+	+	+	+	+		+	0	0	23
Column	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	2±	

The digits represent the highest dilution of the reagents at which agglutination of the cells was visible microscopically. For example, if the first dilution was at 1:90, 1=90, 2=180, . . . , 9=23,040. If two digits are listed, as 8, 9 opposite Pearlneck cells in column 2, there was some variation noted; if 0, ± are listed, it indicates that no agglutination was observed at one or more tests, a faint reaction at others.

Symbols: C=agglutination in one clump; + =variable degree of strong agglutination; ± =weak but definite agglutination; ?=questionable agglutination; 0=no agglutination—all in the first dilution of serum and cells; ②=no agglutination in the second dilution of the serum-cell mixture.

ship was first determined by immunological procedures only, and was later confirmed genetically in that three or more of the unit-characters of Pearlneck (d-6, d-11, and a third component now known to be d-2) segregated according to genetic expectation in the progeny from backcrossing to Senegal the hybrids between Pearlneck and Senegal. (Only two hybrids were raised from this cross. Both were females, and both raised backcross offspring in matings to Senegal, but none in matings to Pearlneck.) As has previously been shown, Senegal cells contain nearly all the antigens *common* to both Pearlneck and Ring dove (IRWIN and COLE 1940), so that Pearlneck and Senegal are more closely related to each other than is either to Ring dove. These three species were at one time assigned to three different genera, but recently PETERS (1937) has placed them all in a single genus, Streptopelia.

The present paper deals primarily with a comparison, by immunological technics, of each of the unit-antigens peculiar to Pearlneck with each of those peculiar to Senegal, and *vice versa*. From the results of such tests one may determine with reasonable certainty which of the antigenic characters particular to these two species are (a) indistinguishable and therefore presumably homologous in the two species, (b) only similar and therefore not entirely homologous, and (c) entirely unrelated. Further, from these relationships one may deduce the similarities and dissimilarities of the genic material producing these cellular substances, by which both Senegal and Pearlneck are differentiated from Ring dove.

Comparisons of the antigenic characters of Pearlneck and Senegal

A first step in making a comparison between the antigenic characters specific to Pearlneck and to Senegal, respectively, is to determine which of the unit-antigens of Senegal are found at least in part in Pearlneck; *i.e.* which cells carrying these substances are reactive with the reagent specific for Pearlneck cells (Pearlneck antiserum absorbed by Ring dove cells). As is given in column 2, lines 14 to 23, of table 1, this reagent agglutinated the cells containing each of the antigenic characters of Senegal (s-1, s-2, s-6, s-7, s-8, s-9, s-11 and s-12) except s-3 and s-10. However, Pearlneck antisera have since been obtained from which reagents have been made which are slightly reactive with the s-3 substance, indicating a relationship of s-3 and some antigenic character of Pearlneck. None has been found, however, which was reactive with the s-10 component. Since the Pearlneck antiserum used in these tests was not reactive with s-3 or s-10 cells, the lack of a reaction obtained whenever these cells were used is not recorded in table 1 (rows 16 and 21).

Likewise, as is given in column 2, lines 4 to 13, of table 2, one or another of the Senegal antiserums—but not all those used in these tests—has been reactive with each of the unit-antigens of Pearlneck. For example, a reagent from one Senegal antiserum might agglutinate d-3 but not d-4, another might agglutinate d-4 but neither d-3 nor d-6, etc. Occasionally a mixture of two or three antisera provided a more satisfactory testing fluid than a single antiserum, but even such a mixture did not always contain antibodies against each of the known antigenic components of either Pearlneck or Senegal.

TABLE 2

A comparison of the cellular antigens specific to Pearlneck and Senegal, respectively, as contrasted with Ring dove, based on their reactions with reagents prepared from Senegal antisera.

CELLS TESTED	ANTI-SENEGAL SERA ABSORBED WITH THE CELLS OF																					
	RING DOVE ONLY	RING DOVE CELLS PLUS THOSE OF																				LINE
		P.N.	d-1	d-2	d-3	d-4	d-5	d-6	d-7	d-9	d-11	d-12	s-1	s-2	s-3	s-6	s-7	s-8	s-9	s-10	s-11	s-12	
Ring dove	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pearlneck	8	0											6		8	8	8	8	8	8	7	8	2
Senegal	9	7,8	7	9	8	8	9	9	9	9	8	9	9	9	9	9	9	9	9	9	9	9	3
d- 1	4,5	0	0	+	+	+	+	+	+	+	+	+	+	②,?	+	+	+	+	+	+	+	+	4
d- 2	4,5	0		0									+	+	+	+	0	+	+	+	+	+	5
d- 3	0,+	0			0									±				±			0		6
d- 4	0,2	0				0				0			+	+		0	±	±			±	±	7
d- 5	7	0					0						0,?	+	+	+	+	+	+	+	+	+	8
d- 6	0,±	0						0															9
d- 7	2	0			?				0				±	+	+	±	±	±	+	±	0	±	10
d- 9	0,2	0				0				0				+		0						±	11
d-11	5,6	±									0		+	+	+	+	+	0	+	+	+	+	12
d-12	0,±	0										0	±	±	±		±	±		±	±	±	13
s- 1	6,7	0,?	+	+	+	+	0,±	+	+	+	+	+	0										14
s- 2	6	②,?	0,±	+	+	+	+	+	+	+	+	+		0									15
s- 3	?,3	?	±	±	±	±	±	?	±	±	±	±			0								16
s- 6	2	0	±	±	±	0	±	±	±	0	±	±				0							17
s- 7	5,6	4,5	+	+	+	+	+	+	+	+	+						0						18
s- 8	7,8	6,7	C	C	+	+	+	+	C	C	+	+						0					19
s- 9	0,+		±	±	±	+	±	±	±	+	+								0				20
s-10	3	+	±	±	±	+	±	±	±	±	±	±								0			21
s-11	0,±	0	±	±	0	±	±	±	0	±	±	±									0		22
s-12	4,5	4	+	+	+	+	+	+	+	+	+	+										0	23
Column	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	

Symbols: same as for table 1.

From these results, it may be stated that each of the antigenic components of Pearlneck is shared in some degree with Senegal, and each of those of Senegal—except s-10—has some relationship with one or more characters of Pearlneck. Further, as is shown in table 1 (column 3, lines 4 to 13), following absorption of Pearlneck antiserum with the cells of both Ring dove and Senegal, antibodies were removed for the cells containing d-1, d-3, d-4, d-5, d-9, d-12 and possibly for d-7, but not for those with d-2, d-6 and d-11. That is, Pearlneck is distinguished from Senegal by a part of the specific characters, d-2, d-6, d-11 and perhaps d-7, in confirmation of previous reports of this relationship (IRWIN and COLE 1940, 1945).

In the reciprocal tests on the unit-antigens of Senegal, using antisera to Senegal exhausted by both Ring dove and Pearlneck cells (table 2, column 3, lines 14 to 23) it is seen that the antibodies were nearly if not completely absorbed for the s-1, s-6, and s-11 substances, possibly for s-2, but not completely for s-7, s-8, s-10, s-12 and probably not for s-3.[3] Thus Senegal is distinguishable from Pearlneck by a part of s-3, s-7, s-8, s-12 and perhaps by all of s-10. One point should be made respecting the reactions obtained following the absorptions of Pearlneck antiserum by Ring dove and Senegal cells. Individual differences in the species hybrids between Senegal and Ring dove have been noted (IRWIN and COLE 1945), explainable by assuming heterozygosity of the Senegal parents for genes effecting substances related to d-1, d-2, and d-4 of Pearlneck. Hence individual Senegals would be expected to vary in content of antigens related to these three substances, if not to more than these three. Such variations have indeed been observed, in that the cells of some Senegals by absorption removed all the antibodies for d-4 of Pearlneck, for example, others removed not quite all, and at the other extreme a few have been noted whose cells removed very few antibodies for d-4, if any at all. The pertinent details of these tests will be reported elsewhere. However, the reactions recorded in table 1, column 3, followed absorptions by Senegal cells which carried as full a content of antigenic components as could be obtained.

It may be noted in table 2 that the reagent (anti-Senegal serum absorbed by both Ring dove and Pearlneck cells) agglutinated the d-11 cells of Pearlneck (column 3, row 12). This seemingly paradoxical reaction may be explained as follows. Data as yet unpublished have shown that Senegal cells are reactive with the reagent used in detecting the hybrid substance (antiserum to the cells of the hybrid between Pearlneck and Ring dove, absorbed by both Pearlneck and Ring dove cells). Since some substance related to this hybrid substance, or to a part of it, is therefore present in Senegal cells, antibodies to them should be reactive with all cells containing this or related components, in this case with d-11 cells. It has been shown (IRWIN and CUMLEY 1945) that the d-11 component of Pearlneck is associated with what is probably the major part of

[3] The reagent used to detect the s-9 character of Senegal was prepared from anti-Pearlneck serum for a majority of the tests with it recorded in tables 1 and 2. Thus it is not possible to state at present whether the cells of Pearlneck would remove the antibodies for the s-9 antigen from Senegal antiserum. Definitely at least a part if not all of s-9 is present in the cells of Pearlneck, as will be discussed later.

the hybrid substance of the species hybrid between Pearlneck and Ring dove, and the reaction of d-11 cells with the reagent resulting from absorption of Senegal antiserum with Pearlneck and Ring dove cells is most probably by virtue of a reactivity with antibodies to the hybrid-like substance in Senegal.

The data in tables 1 and 2 are given primarily to compare the antigenic characters specific to Pearlneck and Senegal, respectively. The reactions in the upper left hand quartile of table 1 would be duplicates of the evidence presented (IRWIN 1939) for the existence as separate entities of the antigenic characters specific to Pearlneck, and are not included, except those which show that d-12 is a different character from the others of Pearlneck. The data in the lower right hand quartile show that the various substances specific to Senegal, as originally detected by reagents prepared from Senegal antiserum (IRWIN and CUMLEY 1947), are also differentiated by the use of Pearlneck antiserum. In table 2, the combinations between reagents and cells of the upper left hand and lower right hand quartiles are not given except to show that the absorption by particular cells was complete. However, when these combinations were tested, no deviation from the expected results was observed.

Certain criteria can be set down to assess the immunological comparisons between the unit-antigens of Pearlneck and Senegal.

1. *Homologous characters:* If an antigenic character of Pearlneck by absorption removed the antibodies for one of Senegal, and *vice versa*, in both anti-Pearlneck and anti-Senegal sera, the two antigens would be indistinguishable and presumably identical, and would be classed as homologous. Such a relationship could obtain only for those antigenic substances of one species which are wholly contained in another, as d-1, d-3, d-4, d-5, d-9 and probably d-12 of Pearlneck which were found *in toto* in Senegal, and the s-1, s-2, s-6 and s-11 antigens, and probably the s-9 of Senegal.

2. *Related but not homologous characters:* If an antigenic character of one species, as of Pearlneck, removes the antibodies for one of the substances of Senegal from Pearlneck antiserum but does not remove the antibodies for that substance from Senegal antiserum, it would be concluded that the substance of Pearlneck was related to, but not identical with, the one of Senegal.

3. *Non-related characters:* If an antigenic substance of one species, as s-10 of Senegal, is not reactive with a reagent specific for Pearlneck components (Pearlneck antiserum absorbed by Ring dove cells); nor do Pearlneck cells by absorption remove antibodies for it), presumably there is no antigen in Pearlneck related to it.

In applying these criteria to proposed immunological relationships, one should always keep in mind the variability of antiserums in their content of antibodies. For example, early tests on the sharing by Senegal of the specific antigenic characters of Pearlneck did not show that only a part of the d-2 character was present in Senegal; *i.e.* absorption of Pearlneck antiserum by Senegal cells removed antibodies for the d-2 antigen (IRWIN and COLE 1940). Another antiserum, however, contained antibodies which, following absorption with Ring dove and Senegal cells, were reactive with cells containing the d-2 substance, establishing the point stated above. From other evidence of this

kind it may reasonably be assumed that each of the cellular characters of this study is quite complex in its antigenic composition; some are more complex than others. If this be true, the antibodies engendered against each antigen in response to immunization will be expected to vary from almost complete absence at one extreme to an antibody against each of the so-called active or determinant groups of the antigen at the other extreme (see LANDSTEINER 1945). Thus, no reagent from a Pearlneck antiserum has yet been obtained which gave more than questionable agglutination with the s-10 component of Senegal. However, if only one Pearlneck antiserum is found with antibodies to s-10, it will establish the fact that at least a part of s-10 is present in Pearlneck, despite all the previous negative findings. Similarly, it is possible that the antigenic characters which appear to be homologous, or nearly so, in the present comparisons might show some differential specificities if other antisera were used.

Cross reactions following absorptions of Pearlneck and Senegal antisera

In table 1 are summarized the cross-reactions of the unit-substances of both Pearlneck and Senegal, following the respective absorptions of Pearlneck antiserum that are listed in the table. The reactions given in the upper left quartile duplicate those previously described (IRWIN 1939), which established the existence of the several substances of Pearlneck as units. As has been stated, the d-4 and d-9 substances are very similar antigenically. In fact, except for differences in association with the so-called hybrid substance (IRWIN and CUMLEY 1945), d-4 and d-9 are practically indistinguishable by use of the antiserums available at the present writing. Also, the d-7 character has been reported to be a complex of two components, of which one is d-3, the other d-7. But the d-7 component has never yet been obtained and perpetuated free of d-3. At the times the tests summarized in table 1 were made, no serum was available which would differentiate the d-7 complex from d-3. Hence the relationships given in the table between d-7 and other characters of either Pearlneck or Senegal duplicate those of d-3. Further, the d-10 substance described in a previous paper (IRWIN and CUMLEY 1945) appears to be the same as d-6, except only that it (d-10) was associated with a fraction of the hybrid substance. The reactions of the d-10 substance as cells or in absorptions have paralleled those of d-6, meaning that these two characters are undoubtedly only one, or d-6.

Homologous antigenic characters

The d-4, d-9 and s-6 characters

There are several cellular characters which behave as units in the respective backcrosses of Pearlneck and Senegal to Ring dove, and which meet the requirements of the criterion given above for the homology of two antigenic substances. Using Pearlneck antiserum, both d-4 and d-9 of Pearlneck by absorption removed antibodies for s-6, but not for any other unit-character of Senegal (table 1, columns 7 and 11, row 17). Likewise, exhaustion by s-6 cells removed antibodies for both d-4 and d-9 characters (table 1, column 17, rows 7 and 11). Further, as is given in table 2, both d-4 and d-9 antigens absorbed

the antibodies from Senegal antiserum for s-6 (columns 7 and 11, row 17) and *vice versa* (column 17, rows 7 and 11). It appears, then, that s-6 of Senegal is indistinguishable from, and therefore presumably homologous to, both d-4 and d-9 of Pearlneck. These results are additional evidence of the close antigenic relationship, if not identity, of the d-4 and d-9 components of Pearlneck.

In a previous report (IRWIN 1939) it was stated that the d-4 and d-9 antigenic characters were tentatively classed as two distinct substances, although differences between them were not always sharply defined and not always present. Also, as is stated above, another possible point of difference was noted later in that, for a relatively small number of offspring of each group, a part of the so-called "hybrid substance" was always associated with the d-4 but not always with the d-9 character (IRWIN and CUMLEY 1945). The backcross birds of the d-9 group, whose cells at one time could definitely be differentiated from those of birds containing d-4, produced no offspring. The cells of offspring from sibs of the birds with d-9 have not been distinguishable from cells having the d-4 character. Hence, although these two specific Pearlneck components are treated separately in the tables, actually they should now be considered as but a single character, which hereafter will be called d-4.

Assuming identity of the d-4 and s-6 antigenic characters, it seems reasonably clear that the one or more genes on a chromosome in each of the two species, Pearlneck and Senegal, which produced d-4 in Pearlneck and s-6 in Senegal, are indistinguishable and presumably identical in the two species. Hence the chromosomes bearing the causative genes may be considered to be homologous, at least from the point of view of the effect on detectable cellular antigens.

There is experimental evidence that both d-4 and s-6 are composed of more than one antigenic substance, in that a fractionation of each has been noted. This would be expected if two or more genes jointly effected the d-4 antigen in Pearlneck and the s-6 in Senegal, and crossing over in a backcross hybrid with a chromosome from Ring dove had occurred. Two backcross birds have been raised whose cells possessed only a part of the d-4 and one with only a part of the s-6 antigen, as compared with the cellular antigen of their respective parents or of other birds giving equivalent immunological reactions.

From matings to Ring doves of backcross hybrids with the cellular character d-4, 106 offspring possessing the character and 101 without it have been produced to date. Routine tests have usually been made on backcross birds with the cellular antigen to determine if the character was the same as that in the cells of the particular parent, or in the cells of some other bird which had given equivalent reactions with the parent. Two (E4OS and E561G) out of 106 have been noted which possessed only a part of the d-4 antigen. That is, the cells of each of these two birds by absorption did not remove all the antibodies for the d-4 antigen, but all the antibodies for the cells of each of these two birds were removed by absorption with other d-4 cells. Direct comparisons of the fraction of the d-4 character which these birds possessed were not possible, because the birds were not alive at the same time. Unfortunately, neither produced offspring. (If it were certain that only two of 106 backcross birds

possessed a fraction of the d-4 character as the result of a crossover, there would appear to be close linkage (c.o. = 1.88 percent) of the causative genes. Unfortunately, not all of these 106 birds were tested for completeness of the d-4 antigen, and not each reagent used in past years was capable of detecting a fractionation. Furthermore, the linkage of the causative genes for the s-6 antigen in Senegal seems to be more easily broken than for the d-4 in Pearlneck. Further studies with present reagents which can more accurately detect the results of crossovers are under way.)

Similarly, a backcross bird (G295E) containing only a part of s-6 has recently been noted. This individual is one of nine backcross birds with s-6, and seven without, from matings to Ring dove of backcross hybrids with s-6 alone or with s-6 and s-7 together. In tests for identity of antigenic composition with the cells of a sib containing the full complement of s-6, and with cells possessing d-4, it was clearly noted that the cells of G295E did not remove all the antibodies for cells with either s-6 or d-4. In contrast, antibodies to the cells of G295E were removed by absorption with cells containing either d-4 or s-6 *in toto*. This bird died without offspring.

The fractionation of an antigenic substance which is homologous in Pearlneck and Senegal might conceivably be explained by assuming mutation of a single causative gene in each kind of backcross progeny. Also if two or more genes are involved in the production of the d-4 and s-6 substances, a mutation in one or more of these genes might have taken place. But the rate of mutation would have been exceedingly high if the fractionation of either d-4 or s-6 would be attributed to mutation. It seems much more reasonable to propose that the d-4 and s-6 substances are produced by the joint action of two or more genes on a single chromosome in each species, and that the three fractionations observed were the result of the separation of these genes as the result of crossing over between the chromosome carrying them and the supposed homologous chromosome of Ring dove.

The d-3, d-7 and s-11 characters

As has been stated (IRWIN and CUMLEY 1945), the d-7 complex of Pearlneck appears to contain the d-3 character as well as what has been termed the d-7. Backcross birds whose cells possessed d-7 without d-3 have never produced any offspring, and were seldom noted. A single backcross male (D458A2) had the combination d-3.d-7 of antigenic components. In successive matings to five Ring doves, over several years he produced 46 offspring, 38 of which had either d-3 or d-7, or both, (the cells of these 38 backcross offspring were agglutinated with the reagent for Pearlneck specific substances) while 8 had neither. These proportions are hardly to be expected if a gene or genes on only one chromosome were involved, but are reasonably close to expectation if there were genes on two chromosomes producing independent parts of the cellular antigens of the parent. Most of these offspring died at a relatively early age, before tests could be made to recognize any which carried only d-7.

In the tests summarized in tables 1 and 2, the cells of the parent bird (D458A2) or of some other which had given equivalent reactions were used in

place of those containing d-7 alone. It may be noted in table 1 that cells carrying d-7 removed antibodies for d-3 (column 10, row 6) but the reciprocal did not obtain (column 6, row 10). Cells possessing either d-3 or s-11 removed the antibodies for each other from either Pearlneck (table 1, column 6, row 22 and column 22, row 6) or Senegal antiserum (table 2, column 6, row 22 and column 22, row 6). These two substances appear to be homologous, and neither removed all the antibodies for d-7 from Pearlneck antiserum (table 1, column 22, row 10, in addition to the combinations given above). However, both d-3 and s-11 removed all or nearly all the antibodies for d-7 from Senegal antiserum (table 2, columns 6 and 22, row 10) suggesting that the antiserums to Senegal did not have antibodies to that part of the d-7 complex which makes it different from d-3. At least a part of d-7 is found in Senegal, since Senegal cells by absorption removed most of the antibodies for d-7 from Pearlneck antiserum (table 1, column 3, row 10).

Since the d-3 and s-11 components are indistinguishable, it may be assumed that they are homologous, or nearly so. Hence the gene or genes which in Pearlneck effect d-3 and in Senegal effect s-11 may likewise be assumed to be homologous. The relationship of the gene or genes which in Pearlneck produce d-7 (apart from d-3) to a gene or genes in Senegal is unknown.

The d-5 and s-1 characters

Both d-5 and s-1 antigenic components were slightly more reactive with the reagents for the specific Pearlneck and Senegal substances, respectively, than were most of the other substances of these two species. Agglutination of each of these substances was noted at a dilution of the respective reagents as high as 1:5,760. Each of these antigenic characters by absorption removed nearly all, if not all, the antibodies for the cells of the other from either Pearlneck (table 1, columns 8 and 14, rows 8 and 14) or Senegal antiserum (table 2, columns 8 and 14, rows 8 and 14). These two substances are very similar antigenically buf seemingly not quite identical.

From these relationships of the antigenic characters, it may be deduced that the gene or genes on a single chromosome which produce d-5 in Pearlneck and s-1 in Senegal are nearly but not quite homologous. If several genes on a chromosome in each species give rise to the specific antigenic character, most of them—but seemingly not all—may be considered to be homologous.

The d-12 and s-9 characters

The only test for the possible identity of these two substances, d-12 and s-9, was that made with Pearlneck antiserum. Each antigenic character by absorption removed the antibodies for the cells of the other from this antiserum (table 1, columns 12 and 20, rows 13 and 20). None of the reagents made from the Senegal antiserums gave strong enough reactions for either d-12 or s-9 to be depended upon to differentiate by reciprocal absorptions between these two antigens.

It seems probable that these two antigenic substances are homologous. On this basis, the causative gene or genes in the two species would also be homologous.

RELATED BUT NOT IDENTICAL ANTIGENIC CHARACTERS

The d-1, s-2 and s-12 antigenic characters

The absorption of Pearlneck antiserum by cells containing d-1 removed all or nearly all the antibodies not only for the s-2 antigenic character of Senegal (column 4, row 15), but for the s-12 character as well (row 23). From these results it appears that the d-1 character of Pearlneck has an antigenic relationship, and therefore a genetic relationship, to both s-2 and s-12 of Senegal. (Only two backcross hybrids with s-2 alone have been available for all the tests described in this paper. Repeated tests on the cells of a dozen or more backcross birds with the s-12 character have always given the same result —*i.e.* no agglutination of s-12 cells following absorption of Pearlneck antiserum with cells containing the d-1 character.)

In the reciprocal tests, using cells containing either s-2 or s-12 in absorbing anti-Pearlneck serum, the absorption by cells with s-2 did not remove quite all the antibodies for d-1 cells (table 1, column 15, row 4). Actually, at one test the reagent resulting from this absorption did not react with d-1 cells, but in each of 11 other tests slight agglutination of the d-1 cells was noted. Following exhaustion of the same antiserum with s-12 cells, there was always strong agglutination of the d-1 substance (column 23, row 4). Hence one may conclude that while the d-1 character of Pearlneck has antigenic relationships to both s-2 and s-12 of Senegal, the majority of the components which make up the d-1 substance are found in s-2, and a relatively smaller proportion in s-12. (All Pearlneck antiserums have contained demonstrable antibodies to the d-1 character and, when tested, to the s-2 character also. In contrast, only about one-third of 23 antiserums to Pearlneck have contained antibodies to the s-12 antigen, indicating that both d-1 and s-2 are more antigenic in immunization than s-12.)

If the d-1 character of Pearlneck is antigenically related to both s-2 and s-12 of Senegal, it would be expected that these two characters when combined in absorption might remove all the antibodies for d-1 cells, or at least more than either alone. [This proposal rests on the assumption that the antigenic constituents of s-2 and s-12 which are related to those of d-1 are different, each from the other. Evidence of the difference between them (s-2 and s-12) is provided by the agglutination of the cells containing each substance following absorption of Pearlneck antiserum by cells containing the other (table 1, column 15, rows 15 and 23; column 23, rows 15 and 23)]. The outcome of four tests following such an absorption is given in table 1 (column 24, row 4). At two of these tests, no definite agglutination of d-1 cells was noted; at two others, only very weak agglutination could be seen. Thus it appears that the combination of the cells containing s-2 and s-12, respectively, removed all, or nearly all, the antibodies for d-1 cells. This result suggests strongly that the substances of s-2 and s-12, similar if not homologous to parts of d-1, are not duplicates but are actually different substances.

Further light on the antigenic similarities of this triangle of cellular characters may be obtained from the reactions following their respective absorptions of antiserums to Senegal cells, as are given in table 2. It will be noted

that the d-1 substance by absorption removed nearly all the antibodies—only weak agglutination was seen—for the s-2 character (column 4, row 4) but diminished very little, if any at all, the agglutinability of the s-12 component (row 23). In the reciprocal tests, using the cells of s-2 in absorption (column 15), all or nearly all the antibodies for d-1 were removed; the absorption by s-12 cells produced no marked change in the reactivity with d-1 cells of the reagent specific for Senegal cells. Hence it appears that d-1 and s-2 are closely related antigenically, and that s-12 has antigenic constituents which differentiate Senegal from Pearlneck, in addition to one or more components related to d-1. The main evidence for the relationship of s-12 to d-1 depends primarily upon the reactivity of s-12 with Pearlneck antiserum.

These several reactions point to the following general relationships between the d-1, s-2 and s-12 characters. The d-1 component behaves in the backcrosses as a single substance; *i.e.*, as if produced by a single gene originally derived from the Pearlneck ancestor, or by two or more genes on a chromosome from Pearlneck with no crossing over having been observed between it and one of Ring dove. In contrast, the s-2 and s-12 characters have segregated, presumably independently, in the backcross generations following the cross between Senegal and Ring dove. (The possibility of loose linkage between the causative genes for s-2 and s-12 will be discussed elsewhere in this paper.) Using Pearlneck antiserum, the d-1 substance removed all or nearly all the antibodies for both s-2 and s-12 antigens. The s-2 character did not remove quite all the antibodies from this antiserum for cells with d-1, and absorption by s-12 had a minimal effect on the agglutinability of d-1 cells. If used together in absorption, these two substances of Senegal removed all or nearly all the antibodies for d-1 cells from Pearlneck antiserum. Thus this antigenic substance (d-1), which behaves as a unit in Pearlneck is seemingly divided into two parts in Senegal (s-2 and a part of s-12).

Although no fractionation of the d-1 substance of Pearlneck has been observed to date, comparable to that described above of the d-4 and s-6 substances, it is probable that two or more genes act jointly to effect this antigen. If this is correct, one would assume that the majority of the genes producing d-1 in Pearlneck have homologues on a chromosome in Senegal, and that these effect s-2. The homologues of the remaining genes would be on another chromosome in Senegal, and would produce that part of s-12 *common* to d-1 of Pearlneck; this chromosome would also carry one or more genes effecting that part of s-12 specific to Senegal. An alternative explanation would be that a single gene in Pearlneck produced d-1, one in Senegal produced s-2, and another produced s-12; on this basis the gene for d-1 in Pearlneck would have an effect similar to two genes in Senegal; for s-2 and for a part of s-12. A combination of these two explanations might also be invoked.

If the genes in Senegal effecting s-2 and s-12 are on independent chromosomes, it means that during the evolution of Pearlneck and Senegal from a common ancestral stock, a chromosomal aberration took place so that the genes (for d-1) on one chromosome of Pearlneck are now on two chromosomes in Senegal. If all the genes which now produce d-1 in Pearlneck were on a

single chromosome in this ancestral stock, the aberration undoubtedly occurred in the pre-Senegal stock so that a separation of these genes was effected as they are now recognized in Senegal. On the other hand, if in this ancestral stock these genes were on two chromosomes, as they now appear to be in Senegal, the chromosomal aberration took place in the pre-Pearlneck stock, and effected a union of genes on one chromosome which now produce d-1.

There still remains the possibility that, in the backcross progeny of the Senegal-Ring dove cross, the two chromosomes carrying the genes for s-2 and s-12, respectively, are the result of a crossover of a single chromosome in the species hybrid. The distribution of these two substances in the species hybrids, and in the backcross offspring of species hybrids which possessed both characters, is not incompatible with that expected of a random segregation of independent chromosomes. However, the number of individuals involved is too small—6 species hybrids and 5 progeny of the first backcross—to settle the point.

The d-2 and s-7 characters

It may be noted in table 1 that absorption of Pearlneck antiserum by cells containing the d-2 antigen removed antibodies for the s-7 character as well as for itself, but not for any other of the specific substances of Senegal (column 5, rows 5 and 18). However, the s-7 character did not exhaust the antibodies from Pearlneck antiserum for the d-2 component of Pearlneck (column 18, rows 5 and 18). On the basis of these reactions these two characters are related, but d-2 has some components specific to Pearlneck not possessed by s-7. Furthermore, when the absorptions were made of Senegal antiserum, the s-7 character removed the antibodies for d-2 (table 2, column 18, rows 5 and 18), but the d-2 antigen did not exhaust the antibodies for s-7 (column 5, rows 5 and 18).

Obviously, the antibodies for d-2 and s-7 are different in Pearlneck and Senegal antiserums. If two or more genes in the respective species by joint action produce the specific antigen, it follows that in Pearlneck at least one gene produces that part of d-2 which is *specific* to Pearlneck, and at least one other gene effects the part of d-2 which is *common* with s-7. Likewise in Senegal at least one gene on a particular chromosome is responsible for that part of s-7 which is *specific* to Senegal, and one or more other genes effect the antigenic component which is *common* with d-2. If but one gene in Pearlneck produces d-2, and another in Senegal effects s-7, each is related to, but not homologous with, the gene in the other species. A combination of these two alternative explanations should not be disregarded, especially if critical evidence could be obtained that several genes on a single chromosome by joint action produced each of these related characters.

The d-11 and s-8 characters

The relationship between the d-11 and s-8 antigens parallels that described above for d-2 and s-7. That is, in using Pearlneck antiserum, the d-11 character absorbed the antibodies for itself and s-8, but s-8 did not remove all those for d-11 (table 1, columns 12 and 19, rows 12 and 19). In parallel tests with Senegal antiserum, d-11 did not by absorption remove all the antibodies for

s-8, whereas s-8 exhausted this serum of antibodies both for itself and d-11 (table 2, columns 12 and 19, rows 12 and 19).

The explanations given above of the genetic relationships between the causative genes for d-2 and s-7 may equally well be applied to those producing d-11 and s-8, except that a gene or genes on a different pair of chromosomes in each species is involved.

The d-6 and s-3 characters

In a previous paper (IRWIN and COLE 1940) it was stated that a reagent made from an anti-Senegal serum was reactive with the d-6 character of Pearlneck. Unfortunately, the Senegal antiserums used in the present tests had no or only weak antibodies for d-6, and the Pearlneck antiserum had only weak antibodies, if any, for s-3 of Senegal. Hence, convincing tests have not been possible of the relationship of d-6 of Pearlneck to s-3 or any of the other unit-antigens of Senegal.

The only suggestion of an antigenic relationship between the d-6 of Pearlneck and s-3 of Senegal is that the absorption of Senegal antiserum by d-6 cells (table 2, column 9, rows 9 and 16) may have removed more of the antibodies for s-3 than the absorption by any other Pearlneck character. On the other hand, reagents for the specific substances of Pearlneck or Senegal, prepared from other antiserums than used in the tables, have not given parallel reactions in the agglutinability of d-6 and s-3, as would have been expected if there were a close similarity in the antigenic constituents of these two characters. Thus it appears that if any antigenic relationship exists between these two specific characters, it is very slight, as would also be the relationship of the respective causative genes in each species. Definitely, however, some antigen in Pearlneck is related in some degree to s-3 in Senegal—therefore genes in each species—since Pearlneck cells by absorption removed some antibodies for s-3 (table 2, column 3, row 16) and several antisera to Pearlneck contained antibodies to s-3.

The s-10 antigen

It is probable that the s-10 character is the only one of the known antigens of Senegal which is truly specific to the species as compared to both Pearlneck and Ring dove. The cells of Pearlneck did not absorb the antibodies for s-10 (table 2, column 2, row 21), and none of many Pearlneck antisera has reacted with the s-10 component. Until a Pearlneck antiserum is obtained with antibodies to s-10, it appears that there is one chromosome of Senegal which bears one or more genes effecting an antigen which distinguishes Senegal from both Pearlneck and Ring dove. No such chromosome is recognized in Pearlneck, for each of the known specific characters of Pearlneck is shared in some degree with Senegal.

Diagrammatic representation of relationships

The relationships between the cellular antigens specific to Pearlneck and to Senegal, respectively, may be represented diagrammatically as in figure 1. Here the antigenic characters in the two species which are presumably identical, or

not identical but related, are given complete or partial cross-hatching in the respective blocks, depending on the relative degree of relationship. Thus, the d-4 and s-6 characters are homologous, or nearly so, and are given complete cross-hatching, as also are d-3 and s-11, d-5 and s-1, and the possibly homologous characters d-12 and s-9. As shown in the diagram, the d-1 component of Pearlneck is made up largely if not entirely of the combined substances—but in quite different proportions of each—of s-2 and s-12 of Senegal; s-12 also possesses one or more parts specific to Senegal.

The antigenic substances of the two species which are related but definitely

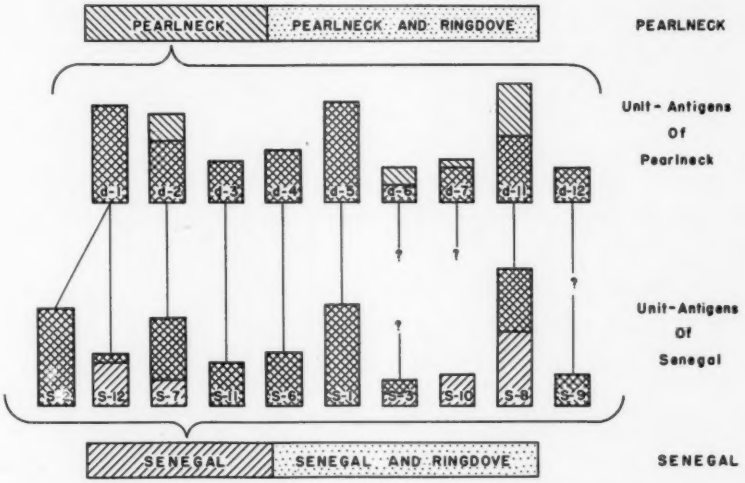

FIGURE 1.—Diagrammatic representation of the relationships of the antigens specific to Pearlneck and Senegal, respectively, in contrast to Ring dove.

not identical in the two species are d-2 and s-7, and d-11 and s-8; the blocks depicting these relationships are only partially cross-hatched. Each of these is probably made up of one or more antigenic components in common with an antigen of the other species, as well as of other components specific to the species from which it was derived. It should be emphasized that the sizes of the respective blocks, and the proportions of the cross-hatched areas in the blocks representing the homologous portions of the related but not identical components, are no more than crude approximations of the quantitative relationships between the respective antigenic characters.

Both d-6 and d-7 of Pearlneck have partial rather than complete counterparts in Senegal, but to which unit character of Senegal each of these is related is unknown at present. It is possible that d-6 and s-3 have antigenic components in common; if this is not the case, the antigen resembling each of these two in the contrasting species probably has not been isolated. Of the specific

antigen s-10 of Senegal, there is no hint of even a related substance in Pearlneck.

As was stated earlier, the finding of any substances of these two species which are indistinguishable and presumably identical, as d-4 and s-6, implies that the gene or genes producing each antigenic character in one of these species have homologues in the other species. It seems reasonable to conclude that the chromosomes in the two species are homologous, at least for genes effecting cellular antigens. Those antigens which show resemblance but not identity, as d-2 and s-7, or d-11 and s-8, require a different explanation. (1) If each related character, as d-2 and s-7, is produced by only a single gene, the antigenic similarity of their respective products would be an index of the similarity but not identity of the causative genes. (2) On the other hand, if two or more genes on a single chromosome by joint action produce d-2 in Pearlneck, and s-7 in Senegal, one or more of these genes in each species could effect the substances *common* in d-2 and s-7,—implying homologous genes—and one or more other genes would then produce the species-specific components of these two related substances. (3) One cannot be certain that the antigenic components which make these two substances alike are identical—*i.e.* they may be only closely related—in which case the causative genes would not be homologous but would presumably resemble each other closely in composition as well as in effect.

DISCUSSION

As has been pointed out, both Pearlneck and Senegal differ from Ring dove in having nine or ten chromosomes which carry genes with effects on antigens not possessed by Ring dove. It is probable that these represent the majority of the chromosomes with genes having at least major effects on the cellular antigens which distinguish either species from Ring dove. If further antigenic differences exist between either or both of these two species and Ring dove, most of them probably are expressed at a lower level of test (*i.e.*, higher concentrations of antisera) than those herein considered. The limits of laboratory technic hardly permit a search for such characters in this material. Undoubtedly, many other genes in these three species than those dealt with in this paper have effects on cellular antigens, and it would be surprising indeed if some of them were not specific to either species. Except for such genes with so-called minor effects on species-specificity, the genes which do not make for species specificity as recognized will produce effects *common* to the contrasting species. However, not much information is yet available concerning the location of the genes producing the cellular antigens *common* to any pair of contrasting species, *i.e.*, whether they are independent of or linked to the genes producing species specific effects. For example, it appears that in Pearlneck and Senegal, there is linkage between genes producing species-specific effects and substances held in common, as the comparisons between d-2 and s-7, d-11 and s-8 have shown.

The only estimate of the number of genes producing cellular antigens common to two species as compared to those effecting the species-specific characters may be obtained indirectly from the relative quantities of cells required in antibody absorption. For example, a given volume of an antiserum to Pearl-

neck at an appropriate dilution might require twice the amount of cells of Ring dove for complete absorption of antibodies for Ring dove cells (2 cc of Ring dove cells: 1 cc of antiserum at 1:60 dilution). Since Pearlneck shares more antigenic substances, and therefore more genes, with Senegal than with Ring dove, it would be expected that more cells of Senegal would be required for complete absorption of antibodies for Senegal cells than of Ring dove for its cells. In actual practice this principle has been found to obtain, since 2.5 to 3 cc of Senegal cells are required in the absorption of 1 cc of anti-Pearlneck serum, as compared with 2 cc of Ring dove cells. But this indirect evidence does not allow more than a statement that it appears highly probable that fully as many, probably more, antigenic characters—therefore their causative genes—are involved in the substances *common* to two species as in those *specific* to each.

Data have been given in a previous report (IRWIN and COLE 1940) which show that nearly all the cellular antigens held in common between Pearlneck and Ring dove are shared also with Senegal. That is, Pearlneck and Ring dove share to the exclusion of Senegal a relatively small proportion of the total antigenic substances. Likewise, Senegal and Ring dove have only a few antigenic effects in common which are not also shared with Pearlneck. Assuming that these general relationships obtain for the causative genes in the respective species, it may be concluded that at least the majority of the genes in Ring dove which produce cellular antigens specific to itself, in comparison with either Pearlneck and Senegal, are therefore in contrast to both these species (IRWIN 1939; IRWIN and CUMLEY 1947). Although these characters have not been obtained as units in backcross generations the available evidence indicates that nine or ten chromosomes of Ring dove are involved in producing effects in contrast to Pearlneck, and possibly about this same number in contrast to Senegal.

The best estimate of the number of chromosomes in these species was obtained by PAINTER and COLE (1943), who found 60-odd chromosomes in the common pigeon (*Columba livia*) and in Ring dove. Of these 30-odd pairs, there are then approximately one-third which carry genes making for differences in cellular antigens between either Pearlneck or Senegal, in contrast with Ring dove. But when Pearlneck and Senegal are compared, there appear to be in Pearlneck only three or four of the chromosomes which carry genes differentiating it from Senegal, and in Senegal there are four or five chromosomes with genes which distinguish it from Pearlneck. Each of the antigenic characters specific to Pearlneck in contrast to Ring dove, is shared to some extent with Senegal. Likewise, all but one (s-10) of the antigenic substances specific to Senegal as compared to Ring dove are shared in some degree with Pearlneck.

Thus it becomes possible to reconstruct with reasonable accuracy the paths which were followed in the evolution of these three species—Pearlneck, Ring dove and Senegal—from a common ancestral stock, by virtue of the genic complexes which effect cellular antigens. What appears to be the most simple pattern of supposed events is as follows. Since Pearlneck and Senegal are more closely related to each other than either is to Ring dove, one may postulate

that the first separation of the original stock was into two populations. One of these became the wild type of the present Ring dove, the other at some later time split again into two populations which are now known as Pearlneck and Senegal, respectively.

It is a matter of speculation as to which of these species has cellular antigens most closely resembling those of the ancestral stock. One might maintain that Pearlneck and Senegal resemble the ancestral stock more than does Ring dove, and that the majority of the genic changes occurred in the Ring dove population. This postulate would rest upon the assumption that Pearlneck and Senegal have had less change in their genic material than Ring dove. Equally probable in the light of our present knowledge would be the argument that the majority of the genic changes took place in the population which gave rise to the Pearlneck and Senegal species, first to differentiate this stock from Ring dove, and then to differentiate Pearlneck from Senegal. It appears more reasonable to postulate that genic changes took place in the several populations which were derived from the common ancestral stock. Also, that the antigenic substances *common* to these three species, Pearlneck, Ring dove and Senegal, represent the effects of genes which have changed slightly if at all from those of the ancestral type.

Recently, statistical analyses have been completed of measurements, made under the supervision of the late L. J. COLE, of several morphological characters of these three species, the species hybrids and backcross hybrids. The details of these analyses will be published elsewhere. In brief it was found that the average value of each character studied—over-all length, extent, length of beak, tail, wing, middle toe, and of the middle toe and claw, diameter of tarsus, and width of neck band—was statistically different from that of the other two species. As would be expected if multiple genes each with small effects were responsible for each morphological character, the average value in the species hybrid was intermediate between the value of the parental species. Likewise, the average values for these morphological characters in the successive backcross generations to either parental species showed a gradual approach to the average measurement of the parental species to which the backcross was made. Definitely, these results conform entirely to the view held by many geneticists, and expressed by MULLER (1940), that such character differences between species depended upon multiple mendelian genes, having individually small effects. The question then presents itself, are the genes responsible for the morphological characteristics of the same sort—not at all implying that the same genes are involved—as those producing the antigenic characters of the blood cells?

The question could be answered in the affirmative if it were found that the Pearlneck species had genes with (small) antigenic effects on chromosomes other than those on which the genes with (large) specific effects are located. But it would not provide information as to why the genes with (major) species specific effects in both Pearlneck and Senegal, in contrast to Ring dove, were located on nine or ten of the thirty-odd chromosomes of these species. This localization on a small number of chromosomes would not be surprising if but

one gene were involved in the production of each substance. However, as was stated earlier, evidence is available that one substance (d-4 in Pearlneck and s-6 in Senegal) in each species is the result of the action of two or more genes, and it seems probable that the majority if not all of the other species-specific substances are also produced by the joint action of two or more genes. Whether or not this statement is accepted, it is reasonable to conclude that the gene or genes in each of these three species responsible for the known species-specific effects are examples of the "gradual increase of the differences between the structures of the genes which had been identical or similar in the ancestral forms," as stated by DOBZHANSKY (1941, p. 85).

SUMMARY

The antigenic characters specific to Pearlneck and Senegal, respectively, in contrast to Ring dove, have been compared for homology or similarity following respective antibody absorptions. The basic assumption is made that those antigenic substances of the two species which are not distinguishable are nearly if not completely homologous, which implies homology of the causative genes. The explanations possible if the substances are related but not identical are discussed.

Nine substances specific to Pearlneck as contrasted to Ring dove were used in these tests: d-1, d-2, d-3, d-4, d-5, d-6, d-7, d-11 and d-12. Similarly, ten substances peculiar to Senegal in comparison with Ring dove were available: s-1, s-2, s-3, s-6, s-7, s-8, s-9, s-10, s-11 and s-12. The following substances were found to be homologous or nearly so in Pearlneck and Senegal: d-3 and s-11, d-4 and s-6, d-5 and s-1, and possibly d-12 and s-9. Those which were related but not identical were d-1 with both s-2 and s-12, d-2 and s-7, d-11 and s-8, and possibly d-6 and s-3. The s-10 cellular character of Senegal seemingly has no related substance in Pearlneck, and therefore appeared to be species-specific to Senegal in contrast to both Pearlneck and Ring dove. Thus, Pearlneck differs from Ring dove by virtue of cellular antigens which are the effects of genes on at least nine of its thirty-odd chromosomes. However, only three or four of these nine chromosomes carry genes (producing d-2, d-6, d-11 and possibly a minute part of d-7) which differentiate it from Senegal. Similarly, Senegal has a minimum of nine or ten chromosomes which possess genes with effects distinguishing it from Ring dove; four or five of these (s-7, s-8, s-10 and s-12 and possibly s-3) set Senegal apart from Pearlneck.

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BIOCHEMICAL MUTANTS IN THE SMUT FUNGUS USTILAGO MAYDIS

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THE usefulness of growth-factor mutants for genetic studies was demonstrated by BEADLE and TATUM in 1941, using the mold Neurospora. Although a number of other microorganisms have since been employed in studies of a similar nature, there has until recently been no instance of the use of biochemical mutants in sexual fungi other than Ascomycetes. This paper is concerned with the production, characterization and inheritance of requirements for essential metabolites in a parasitic basidiomycete, the smut-fungus of corn, *Ustilago maydis* (DC.) Cda. (*Ustilago zeae*, *cf.* STEVENSON and JOHNSON 1944).

The smut-fungi, comprising the order Ustilaginales, are characterized by the production of smut-spores or *chlamydospores* which constitute the zygotes and are typically the only diploid cells in the life cycle. During chlamydospore germination, meiosis occurs in a short hypha, the *promycelium*, from which haploid cells, the *sporidia*, are budded off. In the family Ustilaginaceae these products of the reduction divisions are arranged in a linear order that reflects the events of meiosis. The sporidia multiply saprophytically as discrete, yeast-like cells. In certain environmental circumstances monocaryotic, mycelial growth may occur. (Mycelia may replace sporidia completely in some species, as well as in some variants of species that normally produce sporidia.) Under suitable conditions, haploids of compatible mating-types fuse, giving rise to a dicaryotic mycelium which reproduces by conjugate nuclear division, characteristic of the Basidiomycetes. Formation of a dicaryon is generally essential for virulent infection. In *U. maydis*, though not in many other species, infection is evidently initiated by the haploids and the dicaryon is formed within the host (HANNA 1929). The life cycle is completed by production of chlamydospores within which the two nuclei fuse to form a diploid nucleus. In the case of the maize parasite, this occurs in hypertrophied lesions, the smut-galls. References to the work of mycologists who contributed fundamentally to knowledge of life-cycles of the smuts are given by STAKMAN (1913), and the ecology of the group has been reviewed by TAPKE (1948).

Genetic studies on the Ustilaginales have dealt with the inheritance of factors governing sexual compatibility (*e.g.* KNIEP 1929; BAUCH 1930), colony morphology (*e.g.* DICKINSON 1928, 1931), and pathogenicity (*e.g.* HANNA 1929; ALLISON 1937). Mendelian studies have hardly gone beyond the stage of demonstrating that segregation occurs, and that certain phenotypes can be attributed to single or to multiple factors. Aside from data on the relative fre-

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quency of first- and second-division segregation for a few loci (HÜTTIG 1931, 1933; DICKINSON 1928, 1931), which might be used to establish centromere relations, no adequate information on linkage in any smut has been obtained. This absence of linkage data is particularly surprising since the haploid chromosome number is reportedly two for most species studied (including *U. maydis*, KHARBUSH 1928), a circumstance which would be expected to increase the probability of finding linked characters. The extensive literature on inheritance has been reviewed by CHRISTENSEN and RODENHISER (1940).

In the case of *U. maydis* it has been demonstrated that morphological (STAKMAN, KERNKAMP, KING and MARTIN 1943) and compatibility factors are inherited in a mendelian fashion, that the genetic control of compatibility involves multiple loci (HANNA 1929; CHRISTENSEN 1929, 1931), and that segregants showing different rates of occurrence of morphologically variant colony sectors can be obtained (STAKMAN *et al.* 1943). Segregation of factors governing compatibility and morphological characters may occur at either first or second division (HANNA 1929; CHRISTENSEN 1931; STAKMAN *et al.* 1943). Although virulent infection terminating in chlamydospore formation can ordinarily be obtained only when two haploid lines of compatible mating-type are inoculated together (STAKMAN and CHRISTENSEN 1927), exceptional lines occur in which single sporidial isolates, evidently diploid (CHRISTENSEN 1931; CHILTON 1943; GATTANI 1946), can give rise to infection and spore-formation. While fusion between sporidia from compatible lines has been observed *in vitro* (SLEUMER 1931) the life-cycle of *U. maydis* has never been completed outside the host-plant, as has been possible with several other smut species (see CHRISTENSEN and RODENHISER 1940).

Genetic analysis of *U. maydis* has been impeded by several technical difficulties, most serious of which are the lack of stable, easily characterized differences capable of being used as mendelian markers, the necessity of making crosses in the host plant, and the existence of multipolar sexuality, decreasing as it does the freedom with which segregants can be recombined. The use of growth-factor mutants promises to remove the first of these impediments. There is no reason to believe that proper conditions for making crosses *in vitro* cannot be found. The difficulties imposed by multiple compatibility-factors (themselves of intrinsic interest) can be minimized by identifying the loci concerned and establishing tester stocks.

Certain features of *Ustilago maydis* should amply justify the work involved in overcoming these difficulties. The organism is completely auxoautotrophic (SCHOPFER and BLUMER 1938), requiring no exogenous growth-factors. Haplophase sporidia can be handled like yeasts or bacteria, making possible the application of screening procedures and the quantitative treatment of growth, enzyme adaptation, mutation and selection, as well as facilitating physiological studies (*e.g.* FELDMAN 1948). Sexual reproduction increases the ease of studying biosynthesis and gene-action. Single zygotes can easily be isolated in large numbers prior to germination and the four products of meiosis in each case can be obtained either in order or at random. (The life cycle, even with

crosses in the host, is of about three weeks duration.) The fact that this fungus parasitizes *Zea mays*, which is better known genetically than any other plant, should permit a precise analysis of host-parasite relationships impossible where the host is not controlled. Finally, *Ustilago maydis* represents a major group of sexual fungi as yet little explored genetically.

MATERIALS AND METHODS

Biological Materials

Chlamydospores from a single smut gall obtained in September 1947 from maize growing at Irvington-on-Hudson, New York, were germinated on minimal agar, the sporidia suspended and plated, and stocks made from single colonies picked from plates of suitable dilution. Isolates 4, 5, and 6 were used in subsequent work. DR. E. C. STAKMAN kindly furnished his *U. maydis* stocks 10A4 and 17D4, which were used in preliminary tests of compatibility. An inbred line of maize known to be highly susceptible to smut infection was used for crosses.

Media

The following medium (GRAY and TATUM 1944, modified by addition of trace elements according to RYAN, BEADLE and TATUM 1943) is adequate for growth of non-mutant strains, and will be referred to as minimal medium: NH_4Cl, 5 gms; NH_4NO_3, 1 gm; Na_2SO_4, 2 gms; $MgSO_4 \cdot 7H_2O$, 0.1 gm; $CaCl_2$, trace; K_2HPO_4, 3 gms; KH_2PO_4, 1 gm; Glucose, 5 gms; trace element solution, 1 cc; H_2O distilled, 1,000 cc. The trace element solution is composed of: Boric acid, 114 mg; $FeCl_3 \cdot 6H_2O$, 192 mg; $ZnCl_2$, 840 mg; $MnCl_2 \cdot 4H_2O$, 288 mg; Na_2MoO_4, 84 mg; $CuSO_4$, 750 mg; H_2O, 2 liters. The pH after autoclaving is 6.8.

For solid medium 1.5 percent agar was added. In any experiment where traces of growth-factors might interfere, agar washed fifteen times in distilled water and three times in 95 percent alcohol was used. Minimal medium was supplemented with 1 percent yeast extract (Difco), 1 percent casamino acids (Difco) (acid hydrolysate of casein), 1 percent nucleic acid (Schwarz) plus 2 percent nucleic acid hydrolysate (10 gms yeast nucleic acid autoclaved 20 minutes at 15 lbs. pressure in 50 cc normal NaOH and neutralized), or with individual growth factors as required. Difco "A.C. Broth" (containing, per liter, proteose-peptone, 20 gms; beef extract, 3 gms; yeast extract, 3 gms; malt extract, 3 gms; and dextrose, 5 gms) was in some cases used as complete medium in place of supplemented minimal.

Culture methods

Stocks were maintained on 3 percent agar slants (mortality increases on more moist medium), stored at 3°C, and transferred monthly. Material was grown routinely at 30–32°C. Growth in deep liquid requires aeration; for this purpose a "roller-tube" apparatus of the type used for tissue-culture work (PARKER 1944) was found most convenient. 16×150 mm Pyrex test-tubes

containing 5 cc of liquid medium were placed in fixed position in a drum inclined with axis at an angle of 15° to the horizontal, and rotated at ten revolutions per minute in an incubator maintained at constant temperature. The continuous stirring results in growth comparable to that obtained with vigorous shaking or bubbling. Use of the roller-tubes was suggested by DR. R. BALLENTINE.

Dilutions for plating were made in 0.9 percent NaCl. All plates were prelayered with a small amount of agar to prevent spreading growth at the glass-agar interface.

Turbidometric measurement of growth

A Klett-Summerson photoelectric colorimeter with No. 59 filter and an adapter to take 16×150 mm test-tubes was calibrated against number of cells, using stock 5. Density readings are directly proportional to total cell number over a range from 0 to 250 densitometer units (0 to about 10^7 cells per cc), and then fall off to introduce a 15 percent error at a reading of 360, and progressively greater errors with further increase in density. Since absolute values were not required, growth is expressed in densitometer units.

Ultraviolet irradiation

Freshly grown sporidia suspended in liquid 2 mm deep in 9-cm Petri dishes were exposed to a General Electric 15-watt Germicidal lamp 51 cm distant. Samples of 1 cc, taken at intervals from 0 to 60 minutes after the beginning of the treatment, were diluted and plated in minimal medium.

Detection of mutants

The procedure described by LEDERBERG and TATUM (1946) was used to recover growth-factor deficient mutants from among the survivors of irradiation. This method, applicable to any organism forming discrete colonies in agar medium, allows one to select from a large population any mutant individuals that require an exogenous supply of some water-soluble substance. The detection technique consists of plating the population in minimal agar, which supports development of growth-factor independent colonies but not of mutants devoid of the ability to synthesize some essential metabolite. The latter appear as colonies only after the diffusion of growth-factors from a newly added surface layer of supplemented agar has enabled them to multiply. By varying the composition of the supplement one can select classes of mutants, or mutants with specific requirements.

One cc portions of treated sporidia in various dilutions were pipetted into Petri dishes and thoroughly mixed with liquid minimal agar. After solidification, a thin layer of agar was poured on top to prevent surface growth. The positions of all colonies visible at 48 and 56 hours were marked on the bottom dish with glass-marking ink, and another thin agar layer added containing triple-strength Difco A. C. Broth. Colonies appearing subsequently were picked and transferred to stock tubes of complete medium and tested for growth-factor deficiencies.

Characterization of mutants

The most widely used method for determining requirements of biochemical mutants has been that of inoculating the unknown into a series of tubes containing liquid or solid test media of various compositions. In the present study, two more satisfactory identification procedures were used which involved, in one instance, the spotting of unknown isolates on the surface of agar test-media in Petri dishes, and in the second, the spotting of test substances on a dense minimal-agar plating of an unknown isolate (auxanography). These methods can be employed both for the identification of newly isolated mutants and for the characterization of progeny from crosses.

(1) *Spotting of inocula.* Since *U. maydis* is characterized by localized growth on the surface of agar, it was possible to use, instead of tubes, a series of Petri dishes prepared with test media, and to test up to twenty different isolates on a single plate by inoculating them at marked points. Suspected mutants, obtained from those colonies that appeared after layering with complete medium, were grown on complete slants, suspended, and washed three times in 10 cc portions of saline. A small loopful of each washed culture was inoculated on a series of four plates containing, respectively, minimal agar, minimal+yeast extract, minimal+casamino acids, and minimal+nucleic acid. Those isolates growing only on yeast extract were further tested on plates containing individual B-vitamins (concentrations as used by BEADLE and TATUM 1945); those responding to casamino acids, on individual amino acids (0.2 mg/cc); those showing growth on nucleic acid or both yeast extract and nucleic acid, on individual purines and pyrimidines (1.0 mg/cc or saturated solution).

(2) *Spotting of test-substances (auxanography).* A yet more satisfactory method for characterizing mutants consists of plating dense suspensions of individual isolates in minimal medium and spotting each plate with a series of test-substances. The procedure used with Ustilago is shown in figure 1. A small loopful of sporidia from a freshly-grown (12–24 hr.) slant is suspended in 1 cc sterile saline and pipetted or poured into a prelayered plate to which minimal washed-agar medium is added, thoroughly mixed and allowed to solidify. The test-substances, which need not be sterile, can be spotted on as solid or liquid. With amino acids and nucleic acid components a small particle has generally been used; with vitamins, a loopful of concentrated stock solution. Best results are obtained if plates are incubated 12 hours before spotting. Positive responses are apparent in 24 hours, and are illustrated in figures 3 and 4.

Crosses

Dense suspensions of sporidia from actively growing liquid roller-tube cultures, or from 24-hour slants, were mixed in the desired combinations and inoculated with a hypodermic syringe into maize plants two weeks or more of age, each plant being inoculated at several points at and above the apical bud. Advanced infection, manifested by extensive chlorosis and gall formation, was obtained only at temperatures averaging well above 25°C. Galls appeared after ten days at the earliest, and chlamydospores were obtainable several

days later. Chlamydospores from fresh galls germinate readily, as do those stored for several months at 3°C.

Recovery of products by random isolation

Chlamydospores were suspended in a 1 percent solution of $CuSO_4 \cdot 5H_2O$ for at least 12 hours (CHRISTENSEN and STAKMAN 1926) in order to destroy contaminants or vegetative Ustilago cells, and were then diluted and plated in complete agar. At 32°C chlamydospore germination occurs and promycelia are

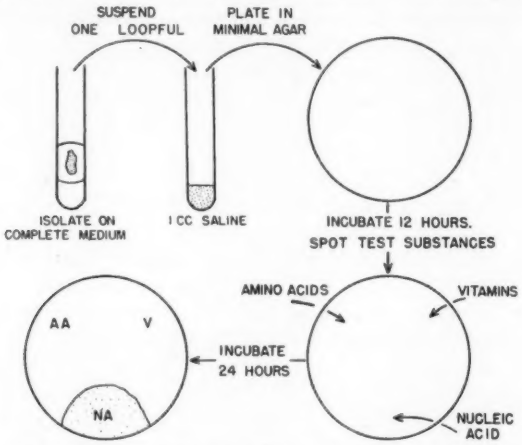

FIGURE 1.—The auxanographic method applied to identification of biochemical mutants in Ustilago. A mutant requiring some component of nucleic acid is illustrated, and specific characterization would be accomplished by spotting a second test-plate with individual purines and pyrimidines.

visible in from 24 to 36 hours. When colonies of sporidia arising from the meiotic products of a chlamydospore (*single chlamydospore colonies*) appeared, whole colonies were cut out individually from plates on which they were widely spaced, and a homogeneous suspension of each was diluted and plated in minimal agar for screening. After non-mutant colonies of unisporidial origin had appeared in these plates, and been marked, the various mutant types were induced to grow either by layering with properly supplemented agar, or by spotting the required substances directly on the agar surface. In the case of crosses involving several loci, segregants can be brought up type by type or all together, as desired. Mutant colonies were transferred to stock tubes and tested auxanographically.

EXPERIMENTAL RESULTS

Description of mutants

Twenty-one isolates unable to grow on minimal medium were obtained from among the sporidia surviving 95 to 99.99 percent killing by ultraviolet irradia-

tion. The specific requirements of fifteen of these mutants were determined by spotting washed inocula of each on the surface of a series of agar plates that contained diagnostic supplements, or by the auxanographic method. (Six mutant isolates have not been fully characterized.) Requirements were rechecked auxanographically, and in most cases by demonstrating a quantitative relation between growth and concentration of the required substance. Mutants were designated by the number of the wild-type sporidial isolate from which they were derived, followed by a specific mutant number; *e.g.*, 5–91 is an isoleucine-requiring mutant obtained following irradiation of sporidia from wild-type stock number 5, which was one of the haploid progeny of chlamydospores obtained from nature. Identified mutants are described in table 1.

All mutants are morphologically and culturally similar to wild-type when grown on optimal medium, with the exception of one yeast-extract requiring isolate which grows as a mycelium and produces no sporidia. All mutants have remained stable through from 4 to 11 monthly transfers on supplemented medium. Ten have been recovered as progeny from crosses (five in two successive generations), demonstrating that they remain stable during several weeks' growth within the host plant. No cases of reversion to growth-factor independence have been observed, except those instances of adaptations in liquid culture noted in table 1.

A number of mutants were grown in roller-tube culture (at $32° \pm 1°C$) and the course of growth on media supplemented with graded concentrations of the required metabolite was followed turbidometrically. Response curves are given in figure 2.

Under conditions where liquid growth exceeded 2×10^7 cells per cc, a rapid decrease in turbidity commenced immediately after maximum concentration was reached, and might deplete the densest populations by one-eighth within 12 hours. For this reason, points on response curves that indicate turbidities of 300 or more are in error by being too low, since readings were taken at intervals of as great as 12 hours, and it is unlikely that the true maximum was recorded. In special cases measurements were rendered inaccurate by pigment-formation or by clotting, but no such difficulty was encountered for the majority of mutants.

The Ustilago mutants so far identified appear to fall into the same categories as biochemical mutants reported in other microorganisms. They can by no means be considered a representative sample of all growth-factor requiring variants, since the type of mutant recovered is a function of the selective screening methods used.

Crosses involving biochemical mutants

Those mutants derived from wild-type isolates 5 and 6 proved to be compatible in crosses with mutant 4–24 and with STAKMAN'S wild-type stock 10A4. Crosses from which first-generation mutant progeny were recovered, either as parental or recombination types, are indicated in table 1. All mutant stocks used in crosses were derived from the same smut gall, and are genetically

TABLE 1

Description of mutants. Under Range of Response is given the highest metabolite concentration supporting negligible growth and the lowest concentration supporting optimal growth. Stability is expressed as the ratio of number of cultures in which adaptation was observed to the total number of cultures in which adaptation could have been detected (tubes containing suboptimal supplements).

ISOLATE NUMBER	REQUIREMENT	RANGE OF RESPONSE	STABILITY IN LIQUID CULTURE	RECOVERED IN PROGENY OF CROSS WITH	REMARKS
5-55	arginine	3×10^{-4}–10^{-1} mg/cc L-arginine	0/28	—	No response to citrulline
5-91	isoleucine	3×10^{-2}–3×10^{-1} mg/cc DL-isoleucine	0/34	4-24 (and in f_2)	Valine without effect
4-24	methionine	10^{-2}–3×10^{-2} mg/cc DL-methionine	1/34	5-4B, 5-25, 5-55 5-76, 5-91, 5-92 5-95, 5-132, 6-A (and in f_2)	No response to cystine, cysteine
5-40	Reduced sulfur		0/13	—	Responds to K_2S, $Na_2S_2O_3$, S-containing amino acids.
5-78	Reduced sulfur		0/6	—	Responds to K_2S, $Na_2S_2O_3$, S-containing amino acids
5-25	adenine or hypoxanthine		18/20	4-24	Order of adaptation is that of decreasing adenine concentration
5-95	adenine or hypoxanthine	3×10^{-4}–10^{-1} mg/cc adenine sulfate	0/42	4-24, 10A4	Slight response to guanine Slight response to guanine
5-132	adenine or hypoxanthine			4 24 (and in f_2)	Slight response to guanine
6 A	adenine or hypoxanthine		4/37	4-24 (and in f_2)	Slight response to guanine
5-92	uracil		6/6	4-24	Order of adaptation is that of decreasing uracil concentration
5-4B	thiamin	$<10^{-4}$–10^{-3} μg/cc thiamin-HCl	0/27	4-24 (and in f_2)	
5-112	thiamin				
5-66	nicotinic acid		10/12		
5-76	nicotinic acid	1.5×10^{-2}–1.5×10^{-1} μg/cc nicotinic acid	0/46	4/24	
4-102	nicotinic acid				

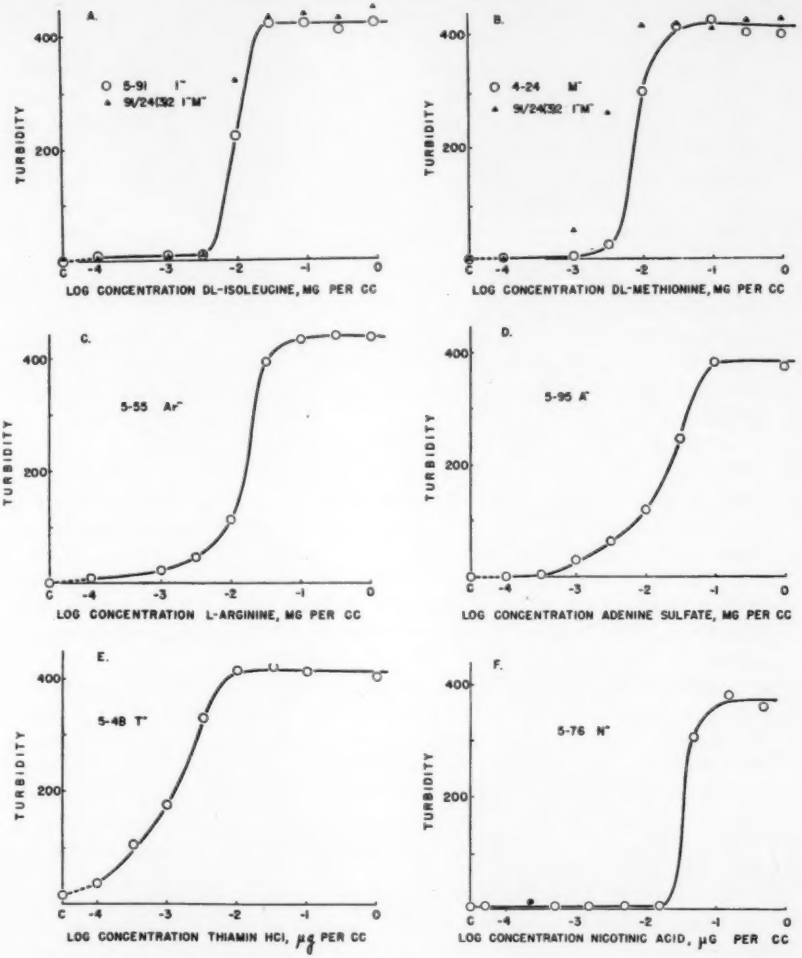

FIGURE 2.—The growth of seven mutant isolates on different concentrations of required substances. Ordinate: turbidity in densitometer units. Abcissa: log concentration of supplement. (C on the abcissa indicates unsupplemented control.) Each point represents the mean turbidity for four tubes at maximum growth or at 230–250 hours after inoculation (except for E where two tubes were used at each concentration).

A. Circles: 5-91 (isoleucineless); triangles: 91/24(3)2 (isoleucineless-methionineless) on minimal medium +0.1 mg/cc DL-methionine. B. Circles: 4-24 (methionineless); triangles: 91/24(3)2 (isoleucineless-methionineless) on minimal medium +0.1 mg/cc DL-isoleucine. C. 5-55 (arginineless). D. 5-95 (adenineless). E. 5-4B (thiaminless). F. 5-76 (nicotinicless).

homogeneous to this extent. (The mutant progeny from 5–95×10A4 were not used in further crosses.)

All four possible types (two parental and two recombinant) were recovered from among the haploid progeny of crosses of 4–24×5–4B, 5–25, 5–76, 5–91, 5–132 and 6A. A number of different double mutants were thus available for use in further crosses. The f_1 isolates were designated by the specific mutant numbers of the parents, separated by a bar, followed by the chlamydospore number, in parentheses, and the f_1 isolate number; *e.g.*, 91/24(3)2 is isolate 2 from a plating of sporidia originating from chlamydospore 3 of the cross 5–91 (I^-M^+)×4–24 (I^+M^-). An isolate bearing this number would be expected to be one of the four types I^+M^+, I^+M^-, I^-M^+, or I^-M^-. This specific isolate proved to be the double mutant.

Characterization of isolates from crosses was accomplished auxanographically, it being possible by this method to determine by use of a single plate which of a number of possible combination-types an unknown segregant represents.

All progeny were obtained from single chlamydospore colonies, by methods described earlier. The type of segregation for individual zygotes could thus be inferred from the kinds of sporidia making up each colony. The three possible types of segregation—$Ab+aB$ (parental ditype), $AB+ab$ (non-parental ditype), and $AB+Ab+aB+ab$ (tetratype) (BURGEFF's terminology, 1929)—were found in one or another dihybrid cross, and are illustrated in figures 3 and 4. The number of tetrads analyzed from individual crosses is not yet sufficient to permit a reliable estimate of the relative frequencies of different segregation types.

Double mutants from among the f_1 isolates were paired in crosses with one another, and galls obtained from a number of combinations. Progeny isolated from single chlamydospores of the crosses 4B/24(7)6 (thiaminless-methionineless)×132/24(2)1 (adenineless-methionineless), and 91/24(3)2 (isoleucineless-methionineless)×6A/24(1)1 (adenineless-methionineless) included all four expected types of individual segregants; tetratype segregations were again encountered while examining the progeny of single-chlamydospores for the presence of triple mutants.

The two triple-mutant and seven double-mutant combinations obtained are indistinguishable from wild-type in the appearance of their growth on complete medium.

DISCUSSION

Auxanography. With Ustilago, the most satisfactory method for detecting and identifying growth-factor requirements has been to observe the response of an isolate to test substances spotted on the surface of a culture densely plated in solid minimal medium. This method is quicker, easier, and less ambiguous than the alternate procedure of testing each culture by inoculating the organism into a series of tubes or plates which contain different supplements. It allows a comparison of responses to different compounds under uniform conditions, distinguishes a specific response from back-mutation, gives a com-

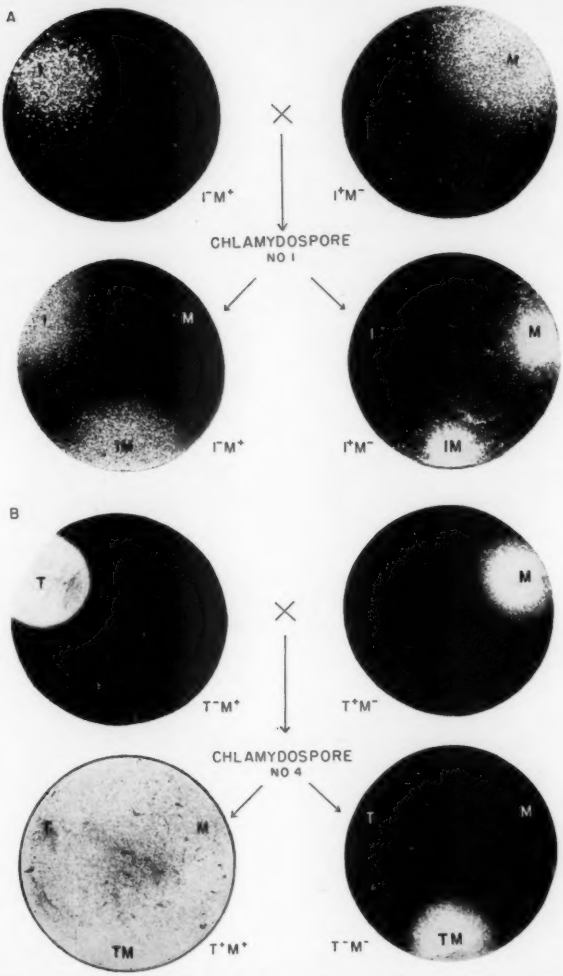

FIGURE 3.—Auxanograms of parents and segregants of single chlamydospores from two crosses.

A. Segregation producing parental types only. Parents: 5–91 (isoleucineless) and 4–24 (methionineless).

B. Segregation producing non-parental types only. Parents: 5–4B (thiaminless) and 4–24 (methionineless). The spots at which test-substances were placed on the plate are indicated by letters on the photographs. I = DL-isoleucine, M = DL-methionine, T = thiamin hydrochloride.

plete concentration-gradient for each substance, permits testing substances of low solubility, and allows testing for single and multiple requirements on the same plate. It can be used to reveal inhibition as well as stimulation of growth.

The surface-spotting of test substances was proposed by BEIJERINCK (1889),

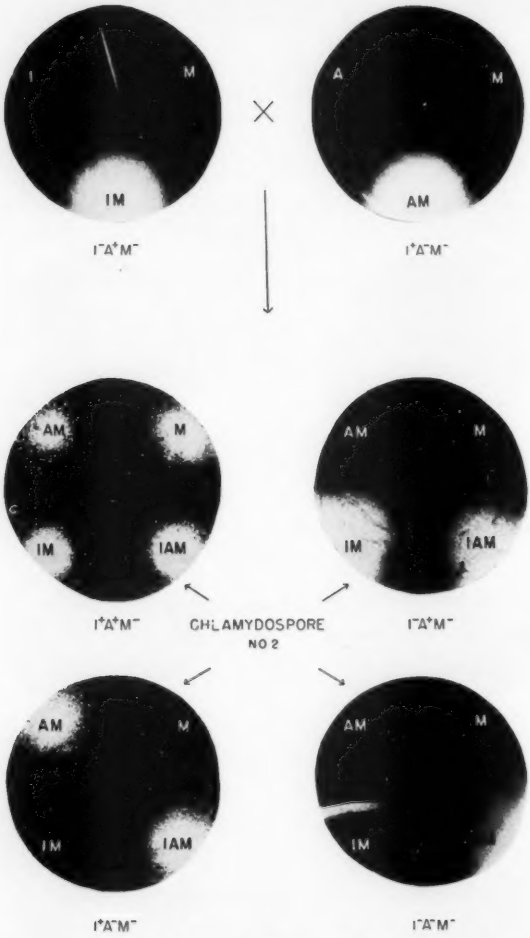

FIGURE 4.—Auxanograms of parents and segregants of a single chlamydospore from a cross of 91/24(3)2 (isoleucineless-methionineless)×6A/24(1)1 (adenineless-methionineless). Segregation produced all four possible types. A=adenine sulfate, I=DL-isoleucine, M=DL-methionine.

who termed the technique *auxanography* and applied it to determine the effect of various substances on luminescence and on the production of acids and pigments. In modified form it has recently been brought into prominence by its use in studies on the effect of antibiotics. Auxanography has been used extensively to test the ability of microorganisms to utilize various carbohydrates and nitrogen sources (*e.g.*, KLUYVER and CUSTERS 1939) and has been adapted to investigating the production of antibiotic-destroying enzymes (WATERWORTH 1948), the effect of inorganic ions (*e.g.*, DUFRÉNOY 1948), and

the effect of substances stimulating sexual-spore formation (HAWKER 1948). Several workers have reported the use of auxanography in studying growth-factor requirements (LUTERAAN and DENIS 1946; LEDERBERG 1946; PONTECORVO 1947; WILLIAMS and PRICE 1948; BACHARACH and CUTHBERTSON 1948; B. DAVIS 1948). At COLUMBIA UNIVERSITY, DR. K. C. ATWOOD has successfully applied auxanographic methods to the characterization of Neurospora mutants, using plated conidia. The manifold applications of BEIJERINCK'S method have been reviewed by DUFRÉNOY (1947).

Growth-factor requirements and parasitism. SCHOPFER and BLUMER (1938) reported that of ten Ustilago species they studied, seven were auxoautotrophic, while the rest required only thiamin or one of its components. This is in surprising contrast to the very exacting requirements of other plant-parasites such as the rust fungi, and raises the question of the relation of growth-factor deficiencies to the parasitic habit.

The fact that combinations of Ustilago mutants, homozygous for an induced growth-factor requirement, are unimpaired in their infective ability suggests that ability to synthesize essential metabolites is probably unrelated to the process of parasitism, but that the selective advantage of growth-factor independence (which must exist to have prevented the accumulation of spontaneously arising deficiency mutations in nature) accrues during some part of the life-cycle that involves saprophytic growth outside the host.

Biochemical mutants as genetic tools. In most discussions of the usefulness of gene controlled growth-factor requirements, the primary emphasis has been placed on their value in furthering knowledge of gene action and biosynthesis. Their importance in this regard has been demonstrated repeatedly, and so successfully that other lines of investigation in which mutants of this type are useful may have been overlooked. Factors governing metabolite requirements can be equally important for biology as tools in the formal genetic study of organisms previously neglected because of technical difficulties. Biochemical mutants are eminently suitable for use as unambiguous genetic markers. They possess a second advantage—the ability to undergo selective screening—in common with but one other category of mutants, those conferring resistance to environmental agents. To illustrate what can be accomplished by employing biochemical mutants as markers, it is only necessary to mention the work of TATUM and LEDERBERG (1947) and LEDERBERG (1947) on sexual recombination in bacteria.

Since BEADLE and TATUM reported the first biochemical mutants in *Neurospora crassa* and *N. sitophila* in 1941, growth-factor mutations have been obtained in *Escherichia coli* (ROEPKE, LIBBY and SMALL 1944; GRAY and TATUM 1944; ANDERSON 1944), *Acetobacter melanogenum*[2] (GRAY and TATUM 1944) *Clostridium septicum* (RYAN, SCHNEIDER and BALLENTINE 1946), *Bacillus subtilis* (BURKHOLDER and GILES 1947), *Bacterium aerogenes* (DEVI, PONTECORVO

[2] Cases where neither the test of sexual recombination nor statistical analysis of the pattern of variation (LURIA and DELBRUCK 1943) have been used to indicate the genic basis of requirement differences are included in this listing because of their resemblance to instances where one or both of these criteria have been applied.

and HIGGENBOTTOM 1947), *Achromobacter fischeri* (MCELROY and FARGHALY 1947), *Azotobacter agilis* (KARLSSON and BARKER 1948), *Absidia glauca* (GILES 1946), *Penicillium notatum* (BONNER 1946; PONTECORVO 1947), *Aspergillus terreus* (RAPER, COGHILL and HOLLAENDER 1945), *Aspergillus nidulans* (HOCKENHULL 1948; PONTECORVO 1948), *Ophiostoma multiannulatum* (FRIES 1945), *Saccharomyces cerevisiae* (REAUME and TATUM 1949; S. POMPER, unpublished), *Coprinus fimetarius* (L. FRIES 1948), and *Ustilago maydis* (this paper). Naturally occurring differences in growth-factor requirements of yeasts have been used by LINDEGREN and LINDEGREN (1947).

No attempt will be made here to deal with the cases where differences in ability to utilize specific energy sources (*e.g.*, WINGE and LAUSTSEN 1939; AUDUREAU 1942; LINDEGREN and LINDEGREN 1946; MONOD 1947; LEWIS 1948, WINGE and ROBERTS 1948; LEDERBERG 1948; EPHRUSSI *et al.* 1949) have been investigated, except to point out that they, like inherited differences in the utilization of nitrogen and sulfur sources (HOROWITZ *et al.* 1945; RAPER *et al.* 1945; FRIES 1946; LAMPEN *et al.* 1947), resemble growth factor mutants, and can be equally useful to the geneticist. Heritable differences of yet another type, affecting chlorophyll synthesis (GRANICK 1948) and photosynthesis (E. DAVIS 1948) have been obtained in Chlorella. The relation of growth-factor mutations to luminescence has been studied by MCELROY and FARGHALY (1947).

Although by far the greatest effort in biochemical genetic studies has been concentrated on *Neurospora crassa* (reviewed by BEADLE 1945), there has already been a considerable extension of similar investigations to other forms. However, the only sexual fungi, aside from Ascomycetes, to which these methods have been applied are Absidia (in which case spore germination could not be obtained), Coprinus, and Ustilago. It seems that the potentialities of biochemical mutants for genetic studies, especially in sexual organisms, have hardly begun to be realized.

SUMMARY

Stable biochemical mutants have been obtained and characterized in the auxoautotrophic smut fungus *Ustilago maydis*, after ultraviolet irradiation. Fifteen isolates requiring specific B-vitamins, amino acids, nucleic acid components, or a source of reduced sulfur, are described.

An auxanographic method has been used for the ready identification and characterization of biochemical mutants.

All possible recombinations of mutant factors were recovered from crosses involving two and three loci at a time. All types of segregations were obtained from single zygotes heterozygous at two loci.

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APPENDIX

Tetrad analysis without knowledge of linearity

Although exhaustive treatment has been given to tetrad analysis where the linear order of segregants is known (MATHER and BEALE 1942; WHITEHOUSE 1942),[3] the more general situation where complete single tetrads are subjected to analysis without consideration of linearity has never received detailed consideration. As genetic studies are increasingly extended to organisms in which the isolation of meiotic products in order is difficult or impossible (Phycomycetes, yeasts, Basidiomycetes, Algae, Bryophytes), mapping procedures will be required that make full use of the information obtainable from single, non-linear tetrads.

In any organism where all four products of a single meiosis can be obtained, it is possible, given any two linked factors, to determine the frequency of recombination between the two, corrected for double-crossovers, by an analysis involving no knowledge of the linear arrangement of segregants. It is further possible, given three unlinked loci, to determine the frequency of crossing over between each locus and its centromere.

A zygote heterozygous at two loci can segregate in any one of three possible ways (table 2). Various chromosomal events and the segregation types to which they give rise are given in table 3.

TABLE 2

Segregation types from a dihybrid cross

TYPE	DESCRIPTION	CONSTITUTION OF TETRAD	
		FOR CROSS $Ab \times aB$	FOR CROSS $AB \times ab$
I	Parental Ditype	$Ab+Ab+aB+aB$	$AB+AB+ab+ab$
II	Non-Parental Ditype	$AB+AB+ab+ab$	$Ab+Ab+aB+aB$
III	Tetratype	$AB+Ab+aB+ab$	$AB+Ab+aB+ab$

For the case where A and B are linked, the frequency of four-strand double-crossovers is that of type II segregations, which give rise only to non-parental types (ignoring multiple crossovers of more than two). Assuming no chromatid interference, the fraction of segregations with no crossovers is given by the frequency of type I minus the frequency of type II segregations,

$$\text{f(non-crossover tetrads)} = \text{f(I)} - \text{f(II)}. \qquad 1$$

Also

$$\text{f(single-crossover tetrads)} = \text{f(III)} - 2\text{f(II)} \qquad 2$$

and

[3] MATHER, K., and G. H. BEALE, 1942 The calculation and precision of linkage values from tetrad analysis. J. Genetics **43**: 1–30; WHITEHOUSE, H. L. K., 1942 Crossing-over in Neurospora. New Phytologist **41**: 23–62.

$$\text{f(double-crossover tetrads)} = 4\,\text{f(II)}. \qquad 3$$

$$\text{Recombination frequency} = \frac{\text{f(singles)} + 2\text{f(doubles)}}{\text{f(non-crossovers)} + \text{f(singles)} + \text{f(doubles)}}$$

$$= \text{f(III)} + 6\text{f(II)}. \qquad 4$$

If frequencies be expressed as decimal fractions,

$$\text{map-distance} = [\text{f(III)} + 6\text{f(II)}] \times 50. \qquad 5$$

This holds regardless of the position of the centromere with respect to the two loci.

For the case where A and B are independent, type III segregations will occur with a frequency equal to $(x-xy)+(y-xy)+1/2xy$, that is,

$$\text{f(III)} = x + y - 3/2xy, \qquad 6$$

where x is the fraction of segregations with crossing-over between A and its centromere, and y the corresponding fraction for B and its centromere (assuming no double-crossovers). By testing both A and B against a third locus independent of both, three simultaneous equations can be obtained and solved for the three unknown recombination-frequencies, each of which is equal to twice the conventional map-distance from locus to centromere.

TABLE 3

The relation of segregation type to crossing-over

TWO LOCI, LINKED		TWO LOCI, INDEPENDENT	
CROSSING-OVER	SEGREGATION TYPE	CROSSING-OVER BETWEEN	SEGREGATION TYPE
No crossing-over	I	Neither locus and centromere	1-I:1-II
Single-crossover	III	One locus and centromere	III
Double-crossovers	1-I : 2-III : 1-II (2-strand:3-strand:4-strand)	Both loci and centromeres	1-I:2-III:1-II

Application to Ustilago maydis. In the corn-smut each colony in a plating of chlamydospores (the zygotes) contains all the products of a single meiosis. Using growth-factor mutants, an auxanogram of the pooled progeny of $Ab+Ab+aB+aB$ segregations will show growth only at the spots where the plate has been supplemented, whereas segregations giving $AB+AB+ab+ab$, or $AB+Ab+aB+ab$, will grow diffusely throughout the plate owing to the presence of growth-factor independent wild-type cells in the population.

For the cross $Ab \times aB$, the frequency of single-chlamydospore cultures giving non-diffuse auxanograms is that of type-I (parental) segregations, while for

AB×*ab*, non-diffuse plates will represent non-parental segregations (type-II). The frequency of type-III segregations can be obtained by subtraction. An equal frequency of I's and II's indicates that *A* and *B* are in separate chromosomes or are separated by at least 50 map-units if on the same chromosome. In cases where I's are more frequent that II's, linkage is indicated, and equation (5) gives the map-distance corrected for occurrence of double-crossovers.

Compared with alternate methods of mapping in Ustilago, this method, whereby a complete tetrad is characterized in a single operation, should be highly efficient. Microdissection is necessary to isolate products in order from the promycelium, and to the difficulty of separating cells whose dimensions are about 10μ is added the problem of irregular formation of promycelia encountered very commonly in some crosses (KERNKAMP and PETTY 1940).[4] Isolation of individual haploid segregants will be necessary for the study of loci incapable of auxanographic characterization, but this method yields no information with respect to biochemically mutant loci that cannot be obtained from pooled tetrads. Such sources of error as selective elimination of the prototrophic haploid type, or early mutation during growth of the mixed colony, must be guarded against.

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THE CYTOGENETICS OF SPECIATION IN GOSSYPIUM. I. SELECTIVE ELIMINATION OF THE DONOR PARENT GENOTYPE IN INTERSPECIFIC BACKCROSSES

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THE cultivated amphidiploid species, *G. hirsutum* L. and *G. barbadense* L., cross readily and the F_1 hybrid shows regular bivalent pairing and is apparently fully fertile. F_2 progenies show considerable depression in vigor, and the net effect of inbreeding is the establishment of types practically indistinguishable from the parent species. All intermediate types are at a great selective disadvantage and fail to establish themselves. A similar situation is found in the hybrid progenies of the Asiatic cultivated diploid species, *G. arboreum* L. and *G. herbaceum* L.

HARLAND'S interpretation of these phenomena (see HARLAND 1933, 1936, 1939) is that the species differences are mainly attributable to differences in "genetic architecture." Through natural selection an integrated system of modifier complexes is built up which is characteristic of each species. When different species are intercrossed, modifier segregation occurs in F_2 and later generations, with a consequent mutual disruption of the two internally balanced parental systems. Species differences on this basis would be independent of structural differentiation of the chromosomes, and the respective genomes could remain cytologically homologous throughout. The regular bivalent pairing and full fertility of the F_1 hybrid has been usually considered to support HARLAND'S hypothesis (SILOW 1941, 1944; DOBZHANSKY 1941; MATHER 1943). For a contrary viewpoint see GOLDSCHMIDT (1940).

In a recent review (STEPHENS 1949b) the writer has suggested an alternative interpretation, and has cited lines of evidence from cytological, genetic and plant breeding sources, which show collectively that something more than multiple gene substitution has been responsible for the differentiation of these species. It has been pointed out that regular bivalent pairing is not a valid criterion of structural homology when the chromosomes are small and the chiasma frequency low. Preferential pairing in synthetic allotetraploids, selective elimination of genes from the donor parent in certain interspecific backcrosses, block transference of linked complexes in others, and the frequency of pseudo-allelic complexes, all suggest that the chromosomes of different species of *Gossypium* have manifold small scale structural differences which are not easily detected by cytological methods. For this type of differentiation, STEBBINS (1945, 1947) has used the convenient term "cryptic structural differentiation."

The evidence for cryptic differentiation is at present only to be found in data

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accumulated for other purposes, and it is important that tests now be set up for the specific purpose of determining its extent and importance in the speciation mechanism. Three possible tests have been briefly indicated (STEPHENS, *loc. cit.*), and it is the purpose of this paper to consider the results of only one of these.

METHODS

If interfertile species have cryptic structural differences it is likely that crossing over between corresponding but only partially homologous chromatids in the F_1 hybrid will lead to the production of gametes containing several small deficiencies and duplications. In Gossypium species the typical situation during meiosis is for two chiasmata to be produced (one per chromosome arm)

TABLE 1

Genetic constitution of parents and tester stocks

Parents				Backcross Testers			
hirsutum		*barbadense*		7 independent loci		5 independent loci	
R_1	Red plant body	r_1	Green plant body	r_1	Green plant body	r_1	Green plant body
R_1^{AF}	Weak spot	R_1^{AB}	Full spot	R_1^{AO}	Spotless	R_1^{AB}	Full spot
p	Cream pollen	P	Yellow pollen	p	Cream pollen	P	Yellow pollen
y	Cream petal	Y	Yellow petal	y	Cream petal	Y	Yellow petal
K	Brown lint	k	White lint	k	White lint	k	White lint
N	Naked seed	n	Not Naked (tufted) seed	n	Not Naked (fuzzy) seed	n	Not Naked (tufted) seed
L^O	Narrow leaf			l	Broad leaf	L^E	Intermediate leaf
Cr^H	"Low" Normal	L^E	Intermediate leaf	Cr^H	"Low" Normal	cr^D	Crinkled
		Cr^B	"High" Normal				

resulting in a ring bivalent at Metaphase I. In interspecific hybrids several of the paired chromosomes only form one chiasma and a rod bivalent results. A type of selection may therefore be envisaged in interspecific backcrosses which would tend to eliminate gametes carrying crossover chromatids, and favor gametes containing non-crossover chromatids. It is likely, too, that non-crossover chromatids of the *recurrent* parent type would have a selective advantage over non-crossover chromatids of the *donor* parent type, since KEARNEY and HARRISON (1932) have shown that selective fertilization occurs in favor of *barbadense* pollen when mixed *barbadense* and *hirsutum* pollen is applied to *barbadense* stigmas, and in favor of *hirsutum* pollen when the mixed pollen is applied to *hirsutum* stigmas. The net effect to be expected, therefore, would be a selective elimination of the donor parent genotype. This may be tested by introducing suitable marker genes into the two species under investigation, making an interspecific hybrid heterozygous for several independent gene pairs, and testing their segregations in reciprocal backcrosses. On the basis of random recombination none of the segregations obtained should differ significantly from a 1:1 ratio but if preferential elimination of gametes occurs the ratios should be skewed due to deficiencies in the classes containing genes from the donor parent.

The stocks chosen for experiment were (1) a line of Upland cotton (*G. hirsutum*) carrying six dominant and two recessive independent marker genes which has been maintained in the genetic collection at this station and (2) an inbred line of Seaberry Sea Island (*G. barbadense*) which carries contrasting alleles to those carried by the Upland stock at the same eight loci. The detailed constitutions of these stocks are shown in table 1. The interspecific hybrid obtained by crossing these two stocks was thus heterozygous for eight marker genes. Ideally the gametes of this hybrid should have been tested by crossing to multiple recessive stocks of both *barbadense* and *hirsutum*. Unfortunately such stocks were not available, and the *hirsutum* stock used (Deltapine 14) only tested seven out of the eight loci. The *barbadense* stock (Sea Island Crinkle) only tested five loci. The detailed constitutions of these tester stocks are also shown in table 1. Backcrosses, *i.e.* crosses to the tester stocks were carried out reciprocally (F_1 ♀ × tester ♂ and tester ♀ × F_1 ♂).

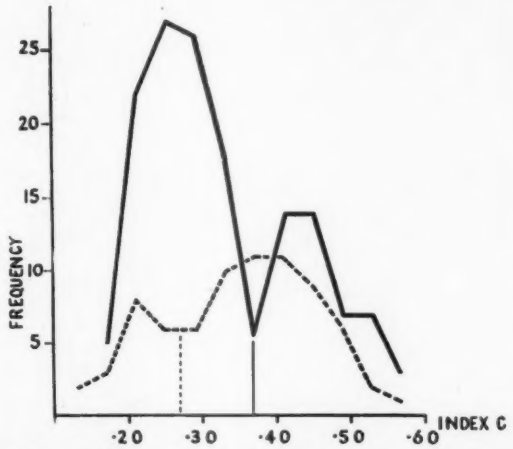

FIGURE 1.—Frequency distributions of leaf shape indices in the backcross, F_1 × *hirsutum* (solid line), and the backcross, F_1 × *barbadense* (broken line). For additional information see text.

No difficulty was encountered in scoring the backcrosses, as the alternative classes were sharply distinct and with the exception of the leaf shape segregations could be scored visually. In order to classify the leaf shape segregations a climax leaf from each plant was measured and its Index C value calculated according to SILOW's (1939) method. Frequency distributions of Index C values for both backcrosses (data from reciprocal backcrosses being pooled) are shown in figure 1. It can be seen that in both cases the frequency distributions are bimodal corresponding to the alternative leaf shape classes expected.

The point of minimum frequency was taken as the point of demarcation between genotypes, and the segregations were classified on this basis as follows:

		Class	*Index C*
(1)	F_1 (L^O/L^E) × *hirsutum* (l)	L^O/l	· · · 0.16–0.36
		L^E/l	· · · 0.38–0.56
(2)	F_1 (L^O/L^E) × *barbadense* (L^E)	L^O/L^E	· · · 0.12–0.26
		L^E/L^E	· · · 0.28–0.56

A word of explanation is necessary about the Crinkle (*Cr*) locus. The alleles, Cr^B and Cr^H both give a normal phenotype but differ in dominance potency (HUTCHINSON 1946a). The heterozygote, Cr^B/cr^D, is phenotypically normal, while Cr^H/cr^D is weakly Crinkled.

RESULTS

The complete data for the backcrosses (F_1×*hirsutum* tester, F_1×*barbadense* tester and the corresponding reciprocals) are given in table 2. The segregations of the different allelomorphic pairs are listed in double columns, the left hand column in each case representing the gene from the *hirsutum* parent.

TABLE 2

Monofactorial segregations in reciprocal backcrosses of the interspecific hybrid hirsutum×barbadense. Alleles from the hirsutum parent are listed in the left hand column in each case.

	R_1	r_1	R_2^{AF}	R_2^{AS}	p	P	y	Y	K	k	N	n	L^O	L^E	Cr^H	Cr^B
(a) *hirsutum* ♀ × F_1 ♂	34	23	39	12	31	25	32	24	22	35	24	33	34	23	—	—
F ♀ × *hirsutum* ♂	54	47	50	38	47	44	48	43	25	63	45	44	69	31	—	—
(a) Combined data	88	70	89	50	78	69	80	67	47	98	69	77	103	54	—	—
Heterogeneity χ^2	0.555		**4.988**		0.191		0.268		1.436		0.994		1.270		—	—
P (1)	.30–.50		.02–.05		.50–.70		.50–.70		.20–.30		.30–.50		.20–.30		—	—
χ^2 (1:1)	2.051		**10.942**		0.551		1.150		**17.940**		0.438		**15.293**		—	—
P (1)	.10–.20		v. low		.70–.80		.20–.30		v. low		.50–.70		v. low		—	—
(b) *barbadense* ♀ × F_1 ♂	3	10	—	—	—	—	—	—	6	6	5	7	6	7	4	9
F_1 ♀ × *barbadense* ♂	37	27	—	—	—	—	—	—	21	36	28	29	13	51	22	42
(b) Combined data	40	37	—	—	—	—	—	—	27	42	33	36	19	58	26	51
Heterogeneity χ^2	**5.215**		—	—	—	—	—	—	0.686		0.221		2.887		0.056	
P (1)	.02–.05		—	—	—	—	—	—	.30–.50		.50–.70		.05–.10		.80–.90	
χ^2 (1:1)	0.117		—	—	—	—	—	—	3.261		0.130		**19.753**		**8.117**	
P (1)	.70–.80		—	—	—	—	—	—	.05–.10		.70–.80		v. low		.001–.01	

Significant differences at 5 percent level shown in bold type. For evidence of allelomorphism of genes listed see HARLAND 1929a (R_1=R and R_2=S in his nomenclature), 1929b (*P*), 1929c (*Y*), 1935 (*K*), 1938 (*N*); STEPHENS 1945 (*L*); HUTCHINSON 1946a (*Cr*).

The backcross, hirsutum tester×F_1, and its reciprocal. The results of this backcross are shown in table 2 (a). Of the seven independent monofactorial segregations tested only one (R_2^{AF}:R_2^{AS}) shows a significant difference between

reciprocal crosses. In this case χ^2 for heterogeneity is just significant at the 5 percent level of probability. Inspection shows, however, that in both crosses there is a deficiency in the R_2^{AS} class, and since the object of the experiment was to test the direction rather than degree of skewness, it seems permissible to pool the data from the two crosses. Considering, therefore, the combined data it can be seen that in five out of the seven segregations tested, the class containing the gene from the *hirsutum* parent is in excess of that expected on a 1:1 basis and in two of these five cases the deviation is highly significant, *viz.* in the segregations $R_2^{AF}:R_2^{AS}$ and $L^O:L^E$. In the remaining two segregations, a reverse situation is found—the classes containing genes from the *barbadense* parent are in excess and in one case ($K:k$) the deviation is highly significant.

The backcross, barbadense tester $\times F_1$, *and its reciprocal.* The results are shown in table 2 (b). Unfortunately only a small family of the cross *barbadense* tester ♀ $\times F_1$♂ was available for study, so that no adequate test could be made for possible reciprocal differences. One significant difference between reciprocal crosses was found, in the segregation $R_1:r_1$, where the ratios are skewed in opposite directions. However, the numbers in the first family are so small that they cannot materially affect the ratios obtained in the second family, so that the combined data may be considered as in the case of the *hirsutum* backcrosses in table 2 (a). Of the five segregations recorded, four show an excess in the class containing the gene from the *barbadense* parent and in two of these the deviation from a 1:1 ratio is highly significant, *viz.* in the segregations $L^O:L^E$ and $Cr^H:Cr^B$.

Comparison between reciprocal crosses in the two backcrosses. Reciprocal differences are only tested adequately in the backcross to *hirsutum*. Nevertheless within the limits of the material studied there seems to be no over-all tendency for selective elimination to be affected by the way in which the cross is made. More precisely there is no consistent tendency for selective elimination to be greater in the F_1 pollen than in the F_1 ovules. This does not imply that gametic selection is unimportant, and in fact there is some evidence that selective elimination in both male and female gametes rather than zygotic selection is mainly responsible for the skewed backcross ratios. It was noticed in making the backcrosses that when the F_1 was used as female about eight to ten percent of the seeds were abortive ("motes"), while when the F_1 was used as male not more than two percent abortive seeds occurred *i.e.* probably not more than would occur by chance in the self fertilized parental species. It is clear that if elimination occurred in the zygote the same proportion of motes should occur whichever way the cross was made, so the most likely situation is that elimination occurs both in the male and female gametes. Elimination in the former cannot be tested directly since there is no adequate technique available for testing the viability of cotton pollen (IYENGAR 1939; STEPHENS 1942) but the absence of heterogeneity in most of the reciprocal backcross segregations does suggest that elimination may be of the same order in male and female gametes.

Comparison between backcross to hirsutum and backcross to barbadense. In

comparing the segregations obtained in backcrosses to *hirsutum* and backcrosses to *barbadense* it will be convenient to consider each allelic pair separately. The segregation $L^O:L^E$ (tables 1 (a) and (b)) gave highly significant deviations from a 1:1 ratio in both backcrosses, and it can be seen that in both cases there is a selective elimination of the allele carried by the donor parent. Thus in the backc oss to *hirsutum* the L^O class is in excess of the L^E class while the reverse situation is found in the backcross to *barbadense*. This reversal shows that the deviations cannot be due to the effects of the alleles themselves, and suggests strongly that there is a selection against the chromosome segment from the donor parent which is marked by the particular locus. In other words it is consistent with the hypothesis that the corresponding leaf shape chromosomes of *hirsutum* and *barbadense* are structurally differentiated. This interpretation is supported by independent evidence (STEPHENS 1949b) that the leaf shape genes are apparently transferred with a block of linked genes in backcrosses of *hirsutum* to *barbadense* and vice versa.

In the case of the $Cr^H:Cr^B$ segregation (table 2 (b)) there is a marked deficiency of the gene from the donor parent in the backcross to *barbadense*. Owing to the absence of a suitable tester stock no comparable data could be obtained in the backcross to *hirsutum*. However, HARLAND's data (1932) do provide this information. They show that in a first backcross of *barbadense* to *hirsutum* of the constitution $Cr^H/cr^D(F_1)\times Cr^H$ (*hirsutum*) the class containing the gene from the donor parent is markedly deficient. It seems likely that the Crinkled chromosomes of *hirsutum* and *barbadense* may also be structurally differentiated. Again this interpretation is supported by independent evidence, as the Crinkled "locus" and the closely linked Corky "locus" are suspected to constitute a complex of pseudo-alleles (STEPHENS 1949a), and the strength of the linkage between Green lint and Crinkled is altered after transference from *barbadense* to *hirsutum* (STEPHENS 1949b).

In the backcross to *hirsutum* (table 1 (a)) the segregation $R_2^{AF}:R_2^{AS}$ shows selective elimination of the gene R_2^{AS} from the donor parent. This situation is in agreement with HARLAND's early data (1929a) where a 4th backcross involving the same locus, *viz.* *hirsutum* spotless$\times$(*hirsutum* spotless$\times$*barbadense* full spot), segregated 579 spot: 706 spotless—a highly significant deficiency of the donor parent class. Unfortunately no comparable data are available for the backcross to *barbadense*.

The only significant deviation from a 1:1 ratio which cannot be interpreted as the result of selective elimination of a gene from the donor parent, occurs in the segregation $K:k$ in the backcross to *hirsutum* (table 1 (a)). Here there is a highly significant deficiency of K types, *i.e.* in this case the gene from the donor parent has an apparent selective *advantage* over its allele from the recurrent parent. No explanation can at present be offered for this phenomenon. The segregating types were readily classified and there appears to be no suggestion in previous literature (WARE 1932; HARLAND 1935; HUTCHINSON 1946b) that the white lint gene *per se* has any selective advantage over its brown lint alleles. In the corresponding backcross to *barbadense*, there is a

tendency which almost reaches significance for the K class from the donor parent to be deficient.

In none of the other segregations studied were there significant deviations from the expected monofactorial ratios, though in the case of $R_1 : r_1$ and $y : Y$ in the backcross to *hirsutum* there is a tendency for the donor parent class to be deficient.

The cumulative effect of selective elimination of the donor parent genotype. Inspection of tables 1 (a) and (b) shows that out of the total of twelve backcross ratios tested nine show a deficiency in the class containing the gene from the donor parent, though in only four of these does the deficiency reach significance. If selective elimination were dependent on structural differentiation, it would seem that only the larger or more extensive structural differences would

TABLE 3

Frequencies of plants carrying various numbers of hirsutum "marker" genes in the backcrosses (a) $F_1 \times$ hirsutum, (b) $F_1 \times$ barbadense.

NUMBER OF *hirsutum* MARKERS	7	6	5	4	3	2	1	0	TOTAL	
(a) Expected $(\frac{1}{2}+\frac{1}{2})^7$	1.09	7.60	22.80	38.01	38.01	22.80	7.60	1.09	139	
Actual	3	9	24	49	32	13	8	1	139	
χ^2	3.35	0.26	0.06	3.18	0.95	4.21	0.02	0.01	12.04	(P(6) = 0.05–0.10)
N.B. Pooling classes with frequencies less than 5 gives $\chi^2 = 9.67$, P(4) = 0.02–0.05										
(b) Expected $(\frac{1}{2}+\frac{1}{2})^5$	—	—	2.16	10.78	21.56	21.56	10.78	2.16	69	
Actual	—	—	1	4	16	25	18	5	69	
χ^2	—	—	0.62	4.26	1.43	0.55	4.84	3.73	15.43	(P(4) = 0.001–0.01)
N.B. Pooling classes with frequencies less than 5 does not alter the probabilities appreciably.										

lead to a significant disturbance in any particular monofactorial segregation In other cases one might expect that the structural differences might be so small that their selective effect would be swamped by random fluctuations, so that they would not be detected in any particular single gene segregation. Nevertheless the cumulative effect of many slight structural differences could be of considerable importance. The easiest way to demonstrate a cumulative effect is to compare the proportion of plants in which the parental genotypes *as a whole* are recovered with the proportion expected on the basis of random recombination. The latter is given by the binomial expansion of $(1/2+1/2)^n$ where n is the number of genetically marked loci. Thus in the backcross to *hirsutum* in which seven loci are marked the number of plants with 7, 6, $\cdots$ 0 independent genes from the *hirsutum* parent should be given by the successive terms in the expansion of the binomial $(1/2+1/2)^7$. The corresponding formula for the backcross to *barbadense* is $(1/2+1/2)^5$. The actual numbers obtained are compared with those expected on the basis of random recombination in table 3 (a) and (b) and in figure 2. It can be seen that in both backcrosses there are significant deviations from the expected values, and inspection shows that in the backcross to *hirsutum* the genes from the *hirsutum* parent are recovered *more* frequently than expected. In the backcross to *barbadense* the

genes from the *hirsutum* parent appear much *less* frequently than expected. The distributions are, therefore, skewed in opposite directions instead of being symmetrically distributed around the median class. Since the loci studied which mark only seven out of twenty-six chromosome pairs exhibit such a marked cumulative effect on the recovery of the parental genotypes, it is clear that the total cumulative effect of the differential segments present throughout the chromosome set must be of a very high order indeed, and certainly sufficient to account for the rapid recovery of the recurrent parent genotype in successive backcrosses which is observed in breeding experiments (KNIGHT 1945).

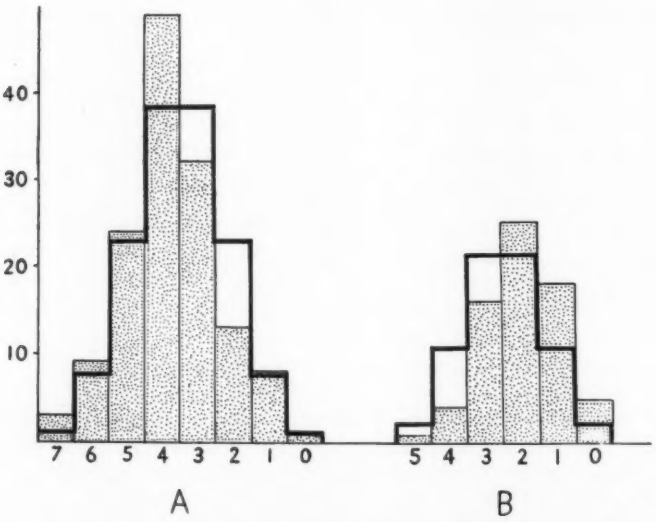

FIGURE 2.—Frequency histograms showing the numbers of plants carrying 0, 1, 2 etc. *hirsutum* marker genes (A) in the backcross to *hirsutum*, (B) in the backcross to *barbadense*. Dotted areas represent the observed frequencies, while the areas enclosed by heavy lines indicate frequencies expected on the basis of random recombination. Data are the same as those shown in table 3.

DISCUSSION

The results of this experiment show that there is a selective elimination of the donor parent genotype in interspecific backcrosses, to whichever parent the backcross is made. The fact that this phenomenon can be demonstrated in small populations in which so few of the chromosomes are marked suggests strongly that the cumulative selective effect on the parental genotypes as a whole must be very great. This agrees with KNIGHT'S (1945) statement that after only two or three backcrosses the progenies closely resemble the recurrent parent. Two obvious questions arise from a consideration of the data. When

is the selection operative—in the gametes or zygotes? What is the mechanism responsible for the elimination?

The fact that very few abortive seeds were found when the F_1 was used as male parent and that practically a complete stand of plants was obtained from the seed collected, shows that zygotic elimination is not an important factor in the first backcross. It might, however, be very important in later backcross generations. About eight percent of all the first backcross plants failed to produce flowers but remained in a vegetative condition. Among the rest of the plants there were considerable differences in time of flowering—some of the plants were harvested before flowering occurred in others. The range of flowering period was much greater than occurs in either parent species. The late flowering types, therefore, in addition to those which did not flower at all, would probably contribute no progeny to the following generation, *i.e.* considerable zygotic elimination would be expected between first and second backcross generations. Returning to the situation in the first backcross, it appears that gametic elimination was of prime importance, and this was confirmed on the female side by the observation that abortive seeds were found when the F_1 was used as female parent, but not when the F_1 was used as male.

With regard to the mechanism responsible for selective elimination, it is clear that the multiple gene substitution hypothesis in its original form is not sufficient to explain the facts. It does not seem to have been realized that the postulation of unlimited numbers of modifiers in an attempt to explain the blurred segregations and depressed vigor in F_2, carries with it the responsibility of explaining their rapid elimination following inbreeding or backcrossing. The more modifiers postulated and the more minute their individual effects, the slower should be their rate of elimination on any selective basis. Another difficulty which does not seem to have been realized is the sharp contrast between F_2 and first backcross segregations. Usually it has been supposed that the relatively slight "modifier disturbance" in the backcross is due to the buffering effect of the balanced genome from the recurrent parent. If, however, the individual modifiers were additive and independent, we should expect that the range of expression of any particular gene in the F_2 would be only halved in the corresponding backcross. In order to obtain a greater contrast between F_2 and backcross it would be necessary to postulate multiplicative interaction between the individual modifiers. This in turn would lead to a violent skewing of F_2 segregations for which there appears to be little evidence. Finally it is difficult to understand how numerous freely assorting modifiers could segregate in such a way as to impart a significant skewness to a monofactorial segregation without blurring the segregating classes.

The first step in the elucidation of these difficulties is the recognition that the "modifiers" are not freely assorting but are carried in internally balanced blocks in each chromosome or chromosome segment. That is, we arrive at MATHER'S (1943) important conception of "polygenic balance," though without being committed to the arbitrary classification of genes into "oligogenes" and "polygenes" which in this connection at least, is not an essential feature

of his theory. It is clear that owing to the low chiasmata frequency in cotton chromosomes these internally balanced complexes could not be broken up effectively in only one generation of backcrossing, and that the chromosomes from the donor parent which carried the complexes (including certain marker genes) might be at a selective disadvantage. Further, any crossing over which did occur in the marked regions would disrupt the balanced complex, and crossover chromatids would also be at a selective disadvantage. Without independent evidence to the contrary, the skewed backcross ratios could therefore be explained by differences based on internally balanced "polygenic complexes" without its being necessary to postulate structural differences. Against this purely genetic interpretation, however, is the independent evidence cited earlier that two of the loci which show the strongest selective elimination are carried on chromosomes which are suspected to be structurally differentiated, and, more generally, the extraordinary rapidity with which the genotype of the donor parent as a whole is eliminated in backcrosses. It is clear that the mechanism of selective elimination would be all the more effective if the "polygenic complexes" were structurally differentiated (*i.e.* were differential segments) since these would have the effect of "locking" the parental gene combinations by reducing crossing over, and any rare crossovers which occurred in their neighborhood would be expected to result in deficiencies and duplications—potentially of far more selective consequence in the gametes than simple genic recombinations. More precise tests for structural differentiation will be considered in later papers in this series.

SUMMARY

(1) Evidence is produced that there is considerable selective elimination of the donor parent genotype in interspecific backcrosses involving *G. hirsutum* L. and *G. barbadense* L. In the first backcross the elimination is primarily gametic and is operative both in pollen and ovules.

(2) The selective elimination can be detected by the significant skewness of specific monofactorial segregations and also by the cumulative tendency for the recurrent parent genotype to be recovered more rapidly than expected as a result of random segregation and recombination.

(3) Out of four loci which showed selective elimination, two are suspected on independent grounds to be carried on structurally differentiated chromosomes.

(4) The results are not explicable by interspecific differentiation based on freely assorting modifier systems, but require some form of internally balanced "polygenic complexes."

(5) Reasons are given for believing that the so-called "polygenic complexes" may actually be structurally differentiated chromosome segments.

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GENETICS Vol. 34 No. 5 SEPTEMBER, 1949

CONTENTS OF PRECEDING ISSUES

NOVEMBER, 1948

COLEMAN, L. C., The cytology of some Western species of Trimerotropis (Acrididae)

CAVALCANTI, A. G. LAGDEN, Geographic variation of chromosome structure in *Drosophila prosaltans*

DOBZHANSKY, THEODOSIUS and HOWARD LEVENE, Genetics of Natural Populations. XVII. Proof of operation of natural selection in wild populations of *Drosophila pseudoobscura*.

GEIRINGER, HILDA, On the mathematics of random mating in case of different recombination values for males and females.

TYLER, W. J. and A. B. CHAPMAN, Genetically reduced prolificacy in rats.

BOURICIUS, JEAN K., Embryological and cytological studies in rats heterozygous for a probable reciprocal translocation.

DOBZHANSKY, THEODOSIUS, Genetics of Natural Populations. XVIII. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographic regions.

Abstracts of papers presented at the 1948 meetings of the Genetics Society of America.

JANUARY, 1949

MULLER, H. J., E. B. Wilson.

HERSKOWITZ, IRWIN, Hexaptera, a homoeotic mutant in *Drosophila melanogaster*.

HAYDEN, BARBARA, and LUTHER SMITH, The relation of atmosphere to biological effects of X-rays.

HERSHEY, A. D., and RAQUEL ROTMAN, Genetic recombination between host-range and plaque-type mutants of bacteriophage in single bacterial cells.

RYAN, FRANCIS, and LILLIAN K. SCHNEIDER, Mutations during the growth of biochemical mutants of *Escherichia coli*.

MARCH, 1949

LURIA, S. E., and R. DULBECCO, Genetic recombinations leading to production of active bacteriophage from ultraviolet inactivated bacteriophage particles.

DULBECCO, R., The number of particles of bacteriophage T2 that can participate in intracellular growth.

RUSSELL, E. S., A quantitative histological study of the pigment found in the coat-color mutants of the house mouse. III. Interdependence among the variable granule attributes.

RUSSELL, E. S., A quantitative histological study of the pigment found in the coat-color mutants of the house mouse. IV. The nature of the effects of genic substitution in five major allelic series.

BAKER, WILLIAM K., The production of chromosome interchanges in *Drosophila virilis*.

MACARTHUR, JOHN W., Selection for small and large body size in the house mouse.

KIMBALL, R. F., The induction of mutations in *Paramecium aurelia* by Beta Radiation.

MAY, 1949

WRIGHT, SEWALL, and ZORA IVASKA BRADDOCK. Colorimetric determination of the amounts of melanin in the hair of diverse genotypes of the guinea pig.

WRIGHT, SEWALL. Estimates of amounts of melanin in the hair of diverse genotypes of the guinea pig, from transformation of empirical grades.

DEMPSTER, EVERETT R. Effects of linkage on parental-combination and recombination frequencies in F_2.

BOZEMAN, M. L., and C. W. METZ. Further studies on sensitivity of chromosomes to irradiation at different meiotic stages in oöcytes of Sciara.

UPHOFF, DELTA E. The expression of alleles at the cubitus interruptus locus in hybrids between *Drosophila melanogaster* and *Drosophila simulans*.

CHASE, SHERRET S. Monoploid frequencies in a commercial double cross hybrid maize, and in its component single cross hybrids and inbred lines.

WARREN, D. C. Linkage relations of autosomal factors in the fowl.

JULY, 1949

BARRATT, R. W., and L. GARNJOBST, Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*.

MERRELL, D. J., Selective mating in *Drosophila melanogaster*.

HAYS, F. A., and D. W. TALMADGE, Inbreeding in a closed flock.

ROBERTSON, ALAN, and I. MICHAEL LERNER, The heritability of all-or-none traits: viability of poultry.

KIMBALL, R. F., Inheritance of mutational changes induced by radiation in *Paramecium aurelia*.

KAUFMANN, B. P., and K. WILSON, Modification of the frequency of chromosomal rearrangements induced by X-rays in Drosophila. IV. Posttreatment with near infrared radiation.

BROWN, S. W., The structure and meiotic behavior of the differentiated chromosomes of tomato.

STRØMNÆS, Ø., The production of dominant lethals with X-rays in aged *Drosophila melanogaster* sperm.

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